April 1961
VOLUME 83/NUMBER 4

THE AMERICAN REVIEW OF Respiratory Diseases

OFFICIAL JOURNAL OF THE AMERICAN THORACIC SOCIETY
Medical Section of the National Tuberculosis Association

1961 ANNUAL MEETING

CINCINNATI, OHIO

MAY 21–25

The 1961 Annual Meeting of the National Tuberculosis Association, the American Thoracic Society, and the National Conference of Tuberculosis Workers will be held in Cincinnati, Ohio, May 21–25.

Registration and the exhibits program will be in the Netherland Hilton Hotel. Program sessions will be held in the Netherland Hilton and cooperating hotels, each within a block of Fountain Square in the heart of the city.

The registration desk will be open beginning at 10:00 a.m., Sunday, May 21. There is no registration fee. A registration badge must be worn to ensure admission to program sessions.

Exhibits will open at 8:30 a.m., Monday, May 22, and will be on view, during morning and afternoon hours, throughout the meeting. The high caliber of exhibits, as at previous meetings, will make this a phase of the program to which all attending will want to devote a generous portion of their time.

Preceding the general meetings—ATS Committee and Council meetings will be held Saturday and Sunday, May 20 and 21. The annual Tuberculosis Nursing Advisory Service Seminar will be held on Saturday, May 20. There will be special interest sessions on Sunday, May 21.

Medical, public health, and nursing sessions will begin on Monday, May 22. General sessions will include an opening program Monday at 9:00 a.m. A keynote, awards, and NTA business session will be held Monday evening, and a closing general session at 11:00 a.m., Thursday, May 25.

Cincinnati is served by five airlines, eight railroads, and three bus lines.

Sleeping rooms for all attending will be available in cooperating hotels within a two-block radius. Application for rooms must be made through the NTA HOUSING BUREAU, 915 Union Central Building, Cincinnati 2, Ohio. Confirmations will be sent directly from the hotel accepting the reservation. If, after making reservations, you find it impossible to attend, please notify the HOUSING BUREAU promptly.

Hotels and rates are:

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Sinton—Single, $6–11.50; Double, $9.50–14; Twin, $11.50–15; Suites, $20 & up.

Official reservation forms will be supplied upon request to the NATIONAL TUBERCULOSIS ASSOCIATION, 1790 BROADWAY, NEW YORK 19, NEW YORK.

APRIL 1961
VOLUME 83/NUMBER 4

THE AMERICAN REVIEW OF Respiratory Diseases

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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ANNUAL MEETING, CINCINNATI, OHIO, May 21–25, 1961

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Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in tuberculosis and other respiratory diseases may apply for membership in the American Thoracic Society. The dues for active membership in the Society are $18 per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

Subscription: The subscription price of the REVIEW is $15 for the calendar year. Checks should be made payable to the American Review of Respiratory Diseases. The price of single copies is $2.00 postpaid.

Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis or respiratory diseases.

MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1955 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title, and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

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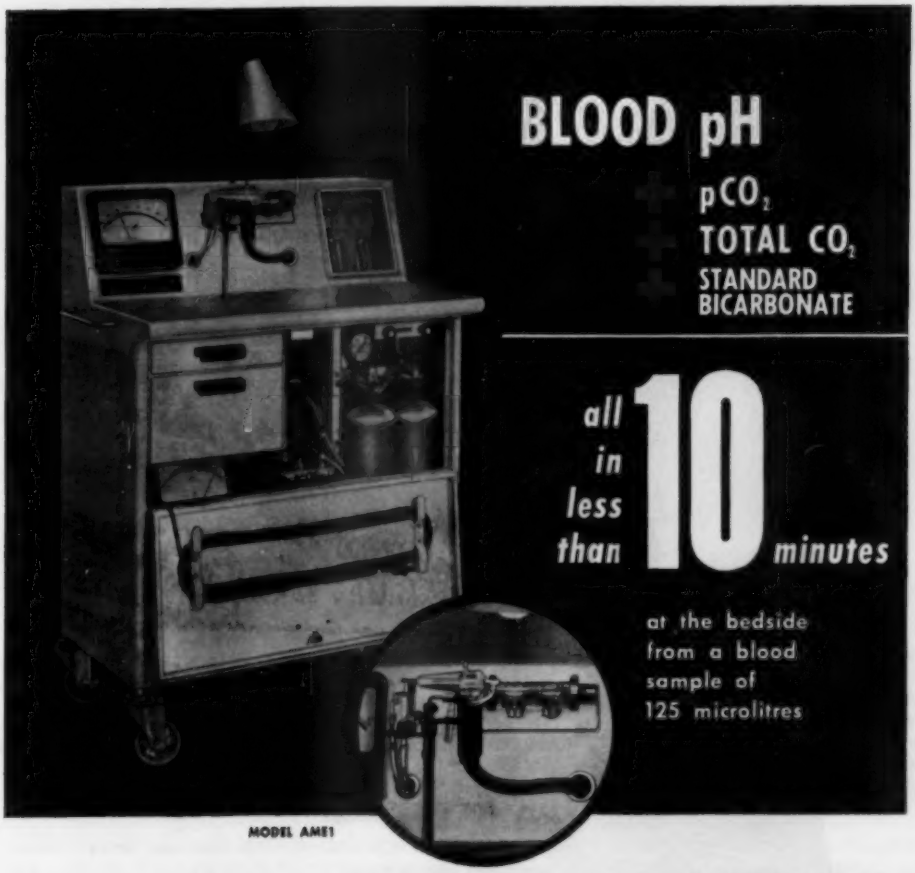

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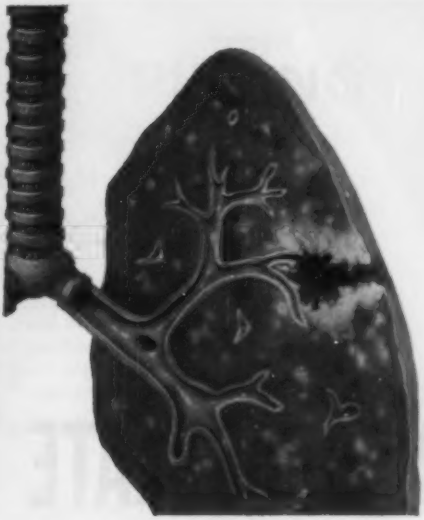

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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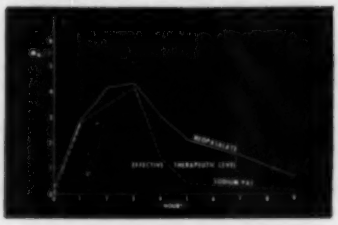

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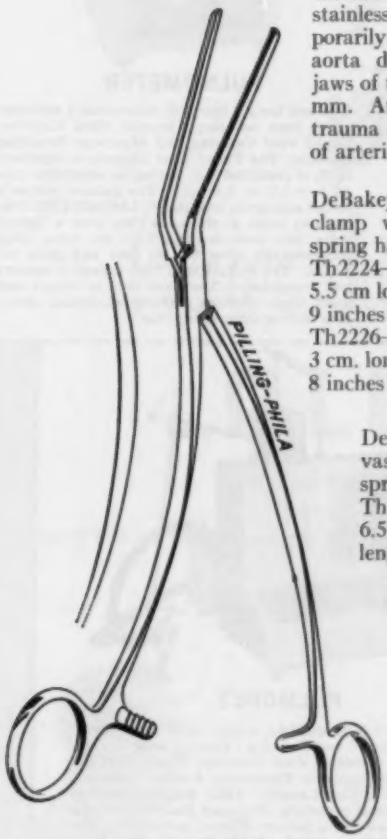

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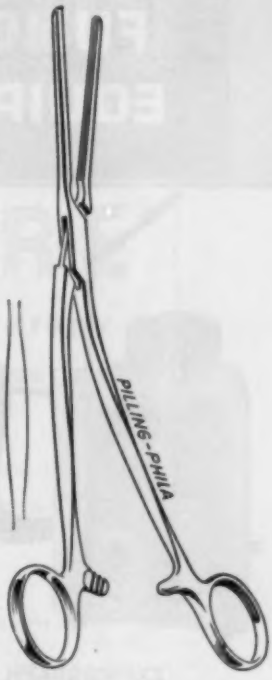

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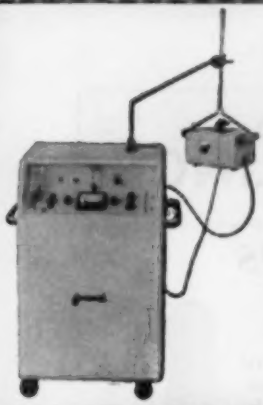

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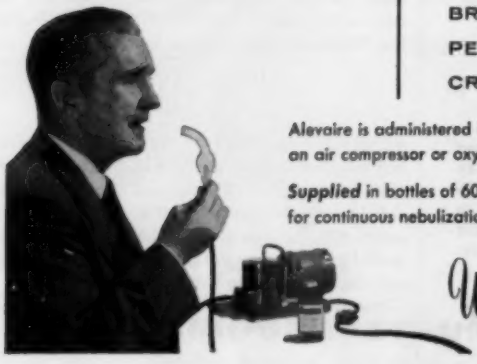

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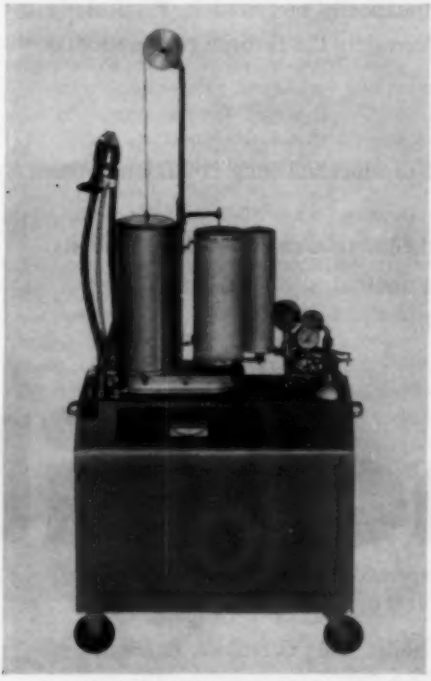

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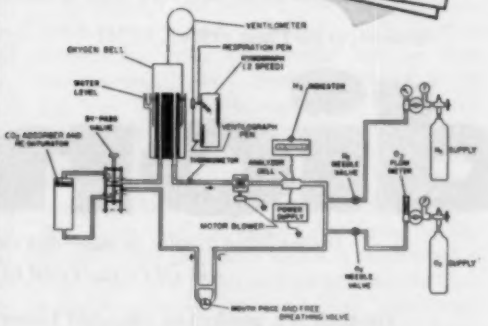

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THE GROWING IMPORTANCE OF PULMONARY HEART DISEASE AS A CAUSE OF CONGESTIVE CARDIAC FAILURE[1,2]

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Chronic cor pulmonale as a cause of congestive heart failure merits particular attention because heart failure due primarily to this cause has become numerically more frequent, and because newer knowledge relative to diagnosis and treatment has become available in recent years.

First, with respect to the etiology and prevalence of pulmonary heart disease, let us consider what factors are responsible for its growing importance as a cause of congestive failure. Chronic pulmonary heart disease, with its characteristic strain on the right ventricle, is a consequence of many different kinds of pulmonary disease of which pulmonary insufficiency is a feature. Not only emphysema, although this is probably the most common cause, but also various pulmonary fibroses and infections, many of them of known specific etiologies, may result in severe pulmonary dysfunction with ultimate development of cor pulmonale. Among these are some diseases, such as tuberculosis and bronchiectasis, from which in the past many, if not the majority, of patients succumbed to the infectious processes per se. Either the toxemia of an infection spreading unchecked or infectious complications, such as meningitis, were the cause of death in many of these patients before pulmonary dysfunction, of such type and severity as to compromise the heart, could develop. With modern effective antimicrobial therapy, most such patients now survive the direct onslaughts of infection, but often not without having sustained crippling damage in the lungs. In a recent survey of the patients hospitalized for tuberculosis in New York City, it was found, for instance, that nearly 500 patients of the total of 3,500 suffered from disabling pulmonary insufficiency (1). Most of these patients would—only a few years ago, before the advent of streptomycin and isoniazid—have died of progressive tuberculosis. Perhaps less definite estimates are possible of the number of patients with bronchiectasis and emphysema who would have succumbed to recurring bacterial infections before the antimicrobial era and died, most probably, of terminal pneumonia. Their absolute numbers must, however, greatly exceed the survivors of tuberculosis.

In England, the registered deaths from bronchitis (and the term bronchitis includes in their classification cases which might here be diagnosed primarily as emphysema) exceed 30,000 per year (2). This mortality rate is approximately twice that of cancer of the lung and four times that of tuberculosis. Of the 30,000 deaths, about two-thirds are listed as due to chronic bronchitis, the remainder being acute or unspecified. I know of no comparable figure in the United States' mortality statistics from which a similar estimate of the proportion of deaths from respiratory causes associated with emphysema and bronchitis may be derived. Chronic bronchitis has been called the "English disease," because of the disparity between the published mortality rates in that country and those published in some other countries, for example, the United States, Canada, and Finland. It seems more likely, however, that the difference is due to the customs and nomenclature of reporting,

[1] From the Department of Medicine, The New York Hospital—Cornell Medical Center, New York, New York.

[2] Presented before the 94th Annual Session, Michigan State Medical Society, Grand Rapids, Michigan, October 2, 1959.

rather than to an actual twenty- to thirty-fold difference in incidence which the published statistics indicate. Precisely what proportion of those deaths listed as bronchitis is due ultimately to heart failure is difficult to estimate from such statistics. The sequence from chronic bronchitis to emphysema to heart failure is now considered commonplace in England, particularly in the industrial areas. In Sheffield, for instance, it was observed by Flint in 1954 that "among the laboring sections of the population chronic pulmonary disease is a commoner cause of heart failure than are coronary disease and hypertension combined" (3). This impression has been recently confirmed in a study of the Sheffield City Hospital and other hospitals in the surrounding area (4). Stuart-Harris and his colleagues found in this study that, in the Sheffield City Hospital, 41 per cent of the cases of heart failure were due to cor pulmonale, compared to 32 per cent caused by hypertensive and ischemic heart disease. In surrounding areas which are in part nonindustrial, the proportion of cases due to cor pulmonale was somewhat less than that attributed to hypertensive and ischemic heart disease, but still comprised 30 per cent of the total. In Sheffield and the other areas combined, cor pulmonale was found to be the cause of congestive heart failure in 35 per cent of 487 patients. I know of no comparable hospital study in the United States. Harrison has noted the increasing proportion of cor pulmonale, although he still ranks this disease as only the fourth or fifth most common cause of congestive heart failure (5).

A somewhat different aspect of the problem was recently studied in Vienna by Popper (6). He found that, in the records of more than 10,000 hospital admissions between 1953 and 1957, slightly more than 2,200 patients had pulmonary emphysema, although in only about one-quarter was this disease the primary diagnosis. In nearly half of the fatal cases of cardiac or circulatory disease other than cor pulmonale, emphysema was present as an associated condition. In contrast, emphysema was associated with only 20 per cent of other fatal diseases. This indicates the significance of an associated emphysema in the development of cardiac decompensation in patients with primary heart disease. Of particular interest is the finding that, among patients with myocardial infarction, the mortality was more than twice as high in patients with emphysema as in those without. A similar relation was found among cases of pulmonary embolism, as well as of valvular heart disease. In these patients also, the mortality was more than twice as high in those with associated emphysema as in those without. Thus, not only as an independent cause of heart failure, but as a contributory cause when the heart is otherwise diseased, pulmonary heart disease emerges as a major factor in the etiology of congestive heart failure.

The growing recognition of the high prevalence of pulmonary heart disease undoubtedly reflects in part an absolute increase in crippling pulmonary diseases, for reasons already mentioned. There is also, however, the factor of improvements in diagnosis. These stem in large part from the recent advances in cardiopulmonary physiology and improved methods of function testing including, of course, cardiac catheterization. Indeed, according to Fishman and Richards the earliest evidence of cor pulmonale secondary to chronic pulmonary disease can be obtained only by cardiac catheterization (7). There are, however, clinical features of pulmonary hypertension and cor pulmonale with and without right ventricular failure which may be recognized, if not in the incipient stage, at least when the condition is still amenable to therapy.

An understanding of the diverse physiologic states which pertain to the pathogenesis of cor pulmonale is essential to an appreciation of the clinical features which must be looked for. Blood gas analyses, cardiac catheterization, and other physiologic investigations are not available for every patient, nor indeed are they always necessary for clinical diagnosis. By relating observations obtainable by such special methods to clinical signs and symptoms, however, the significance of the latter and their limitations in diagnosis may be better appreciated. In certain situations, moreover, precise observations in the cardiopulmonary laboratory are indispensable.

Pulmonary heart disease which results, as it does, from primary disease of the pulmonary parenchyma or pulmonary vasculature is essentially a disease of the right heart. The cardinal physiologic features are dilatation, hypertrophy, or failure of the right heart as a consequence of hypoxia and pulmonary hypertension. By definition, however, the term cor pulmonale (or pulmonary heart disease) is excluded when such a disorder of the right heart is secondary to dis-

ease of the left heart—as in rheumatic heart disease with mitral stenosis—or to congenital cardiac anomalies.

A division into hypoxic and hypertensive cor pulmonale has been made on the basis of which of these functional disorders predominates (8). The hypoxic type thus includes cor pulmonale secondary to chronic emphysema (sometimes called obstructive emphysema), to pneumoconiosis, bronchiectasis, tuberculosis, Hamman-Rich disease, sarcoidosis, and the hypoventilation syndrome. Carbon dioxide retention may or may not be present in the hypoxic type, depending on whether poor aeration of perfused alveoli is the predominant defect, as in severe chronic obstructive emphysema, or whether the defect is one primarily of impaired diffusion across the alveolar-capillary membrane, as occurs in certain of the granulomatoses and fibroses (most characteristically, perhaps, in the late stages of beryllium disease and in the Hamman-Rich syndrome). The pulmonary hypertensive type, in this division, includes primary pulmonary hypertension and secondary pulmonary hypertension due to such causes as pulmonary embolism (particularly multiple embolizations), collagen diseases affecting primarily the pulmonary vasculature (polyarteritis nodosa and scleroderma have been cited as examples, but these may also cause parenchymal fibrosis), pulmonary schistosomiasis, and metastatic carcinomatosis. The principal value of this division is perhaps to emphasize that, in the pulmonary hypertensive type, hypertension of a very high degree is commonly observed. In contrast, in the hypoxic group, the pulmonary hypertension is usually of lesser degree and usually not severe enough to cause right ventricular failure of itself without the additional factor of anoxemia and its effect on the myocardium. However, the polycythemia and the anoxemia per se tend to increase pulmonary hypertension, which may be of extreme degree in a proportion of this group also. These consequences of anoxemia are the essential mechanisms whereby failure may be precipitated when acute respiratory infections cause a sudden fall in arterial oxygen saturation.

If special investigations are available, the most important in a case of suspected cor pulmonale secondary to emphysema is a determination of the arterial oxygen saturation. This is always below the normal level in cor pulmonale, and circulatory changes of increasing pulmonary arterial pressure and cardiac output occur in pulmonary insufficiency when the saturation falls below 90 per cent (9). If the arterial oxygen saturation is below 85 per cent (according to Fishman and Richards) and if, in addition, there are marked polycythemia and carbon dioxide retention, a primary pulmonary etiology of heart failure is probable (7). Such a degree of oxygen unsaturation, namely, below 85 per cent, becomes detectable clinically in the form of so-called "central" cyanosis of the tongue, the lips, and the conjunctivae. In the aforementioned study in the Sheffield area, central cyanosis at least of the lips was present in 93 per cent of the cases of cor pulmonale. Since some patients with cor pulmonale due to emphysema have saturations between 85 and 90 per cent, the absence of a bedside demonstration of cyanosis does not exclude this diagnosis, and it is for this group that blood gas analysis for oxygen is indispensable. Measurements of carbon dioxide content and partial pressure in the blood, as well as a determination of pH, are necessary to fully evaluate the degree of carbon dioxide retention and respiratory acidosis. However, measurement of the alkali reserve, which is secondarily affected in hypercapnia by a retention of bicarbonate by the kidney, is generally used as a substitute, albeit an imperfect one. In the absence of a metabolic acidosis from another cause, carbon dioxide retention will be reflected in an elevation of carbon dioxide combining power.

The presence of carbon dioxide retention is a particularly common feature of pulmonary insufficiency due to emphysema. A most important consequence of this retention is the impairment of the sensitivity of the respiratory center. Diagnostically, its presence in heart failure supports other evidence that may point to cor pulmonale. It indicates further a predominance of an alveolar-respiratory defect, in contrast to the alveolar-capillary block type of defect in which carbon dioxide content is characteristically normal or low. In addition to its diagnostic importance in these respects, a direct or an indirect estimation of the carbon dioxide content of the blood is of value as a guide to therapy, with special reference to the administration of oxygen and the well-known dangers of continuous high-concentration oxygen therapy in respiratory acidosis.

Cardiac catheterization is the least readily available of the special methods of study, but it

has added greatly to the understanding of the various mechanisms which may cause cor pulmonale and, ultimately, heart failure. Not only can the pressure relationships be determined, but also the cardiac output and the presence or absence of shunts, which may be of diagnostic importance, particularly in differentiating pulmonary hypertension secondary to congenital defects from that secondary to pulmonary disease. In chronic pulmonary disease, the primary cause of pulmonary hypertension is reduction in the pulmonary capillary bed. The extent of structural changes in the pulmonary vasculature varies in different forms of pulmonary disease. It is usually less in chronic obstructive emphysema, for instance, than in certain other conditions with greater destruction of vessel-bearing lung tissue. Whether in bronchitis and emphysema, however, or in cor pulmonale associated with other primary lung conditions, it has been shown that pulmonary hypertension is always present when there is failure (10).

The interrelationships between pulmonary hypertension, cardiac output, hypoxia, and polycythemia are complex. The pulmonary vascular resistance, which is a derived measure usually expressed in units, is calculated from the mean pulmonary artery pressure, the mean pulmonary capillary pressure (which approximates left atrial pressure), and the cardiac output. The cardiac output usually bears an inverse relationship to the pulmonary vascular resistance. If the resistance is high, the cardiac output is low. The signs, then, are essentially those of pulmonary hypertension, and include a small pulse, peripheral vasoconstriction, a large "a" wave in the venous pulse, increased right ventricular pulsation, a markedly accentuated pulmonary second sound and, often, a pulmonary diastolic murmur. If the resistance is low, the cardiac output is high and the clinical signs are those of a hyperkinetic circulatory state. This includes warm extremities, water-hammer arterial pulse, capillary pulsations, slight-to-moderate elevation of venous pressure, sinus tachycardia, and a gallop rhythm. These signs may persist even with the development of congestive failure although, when this is advanced, cardiac output usually falls. Congestive failure itself is, of course, accompanied by the usual signs of increased venous pressure, edema, liver enlargement, functional tricuspid insufficiency, and the like. In the presence of emphysema, the auscultatory signs of pulmonary hypertension may be obscured either in the absence or presence of congestive failure.

The electrocardiogram offers less assistance in the diagnosis of cor pulmonale than is generally supposed. This is partly because it is difficult to separate those changes which may be due to emphysema alone—such as the signs of clockwise rotation—from those which indicate right heart hypertrophy. Moreover, the latter may not appear until late in the disease, or they may not appear at all even in severe cor pulmonale with extreme pulmonary vascular resistance. A low incidence of arrythmias, particularly atrial fibrillation, is well known.

Similarly, roentgenographic examination of the chest is not necessarily helpful, and may be confusing—as when a very prominent right pulmonary artery may simulate a tumor. The cardinal roentgenographic signs of pulmonary hypertension are well known, with dilation of the main branches of the pulmonary artery and attenuation of the peripheral branches. But these signs are more obvious in the absence of heart failure than when this complication supervenes. Right heart enlargement, contrariwise, may not be appreciable roentgenographically until failure is advanced. Suggestive or definite signs of emphysema, or of other pulmonary disease, may point to the diagnosis of pulmonary heart disease. With respect to emphysema, however, it has been argued whether there are any truly confirmatory roentgenographic signs, and that voluminous lungs are not necessarily emphysematous lungs. Indeed, if one wishes to go this far back into etiologic factors, there is neither unanimity on a definition of emphysema nor agreement on its pathogenesis. Nor is it at all clear why some patients with extensive bullous emphysema, for instance, have no symptoms and little demonstrable evidence of pulmonary dysfunction, while others may progress rapidly to extreme pulmonary insufficiency and cor pulmonale. A final point is that many patients with severe pulmonary disease and pulmonary insufficiency, even with pulmonary hypertension, do not develop cor pulmonale or, at least, not to the extent of circulatory failure.

It has been shown, however, by Stuart-Harris and his co-workers, that patients with chronic bronchitis and emphysema without heart failure may have renal ischemia during exacerbations of bronchitis, followed by improvement in the renal circulation during the recovery

phase (11). These changes are similar to the renal circulatory changes which occur in cor pulmonale with cardiac failure, which also are reversible. This is in contrast, moreover, to the renal circulatory impairment in most forms of heart failure, which tends to be irreversible once it is established. Circulatory changes in pulmonary insufficiency probably occur considerably more often than is recognized. Perhaps it is on this account, rather than its effect (if any) on reducing hypercapnia, that long-term therapy with acetazolamide (Diamox®) is often found to be symptomatically beneficial in chronic emphysema.

The therapy in cor pulmonale must be considered not only with regard to correction of the circulatory dysfunction, but to treatment of the pulmonary insufficiency and the underlying pulmonary disease. With respect to the management of congestive failure itself, the former notion that digitalis is ineffectual in heart failure due to pulmonary heart disease has, of course, been dispelled in recent years. Congestive failure in cor pulmonale is, therefore, treated in this respect much as that resulting from any other cause. Diuretics are also used in much the same way as in other forms of heart disease, together with a low salt intake. Acetazolamide, which is but a relatively weak diuretic, is recommended by Richards and Cournand and their associates more for its effect in reducing a persistent elevation of carbon dioxide, when this is present, than primarily for its diuretic effect (7, 12). This is, however, not negligible in my own experience, although diuresis is not so rapidly accomplished as with mercurials or chlorothiazide.

In addition to the usual cardiotonic regimen for heart failure, it is essential that the underlying pulmonary condition be adequately treated. This involves antimicrobial therapy for bronchitis or other pulmonary infection which, particularly in chronic obstructive emphysema, is often the precipitating cause of respiratory failure, hypercapnia, and heart failure. Overt signs of infection may be inconspicuous or absent, but these patients should nevertheless receive broad-spectrum antimicrobials. Bronchodilator aerosols may also be indicated to relieve bronchospasm and, particularly in patients with long-standing asthma, the corticosteroid hormones may help to reduce secretions and relieve airway obstruction. Tracheal suction may be indicated and, in some instances, tracheostomy is required to permit adequate ventilation.

The most difficult problems are those connected with the relief of hypoxia and, simultaneously, carbon dioxide retention when this is severe. Here one may encounter the dilemma of having to restrict oxygen therapy in order to avoid aggravating the condition of hypercapnia and, possibly, causing apnea by removal of the hypoxic stimulus to respiration by the administration of high oxygen concentrations. The concern in this regard has sometimes become exaggerated to the extent that adequate oxygen therapy may be withheld, to the detriment of the outcome. Hypoxia, because of the very nature of the disease, is almost always the primary problem. In certain forms of pulmonary disease which lead to cor pulmonale, particularly in those characterized as the alveolar-capillary block syndrome, hypercapnia is usually not present and oxygen not only may but should be given in high concentration. Relatively high oxygen mixtures may also be given with or without mechanical aids to ventilation when hypercapnia is not extreme. Caution in the use of oxygen must be exercised, however, in the patients with carbon dioxide retention of considerable degree. The practices of administering oxygen by nasal catheter to avoid excessive concentrations, or intermittently in higher concentrations, still have much to recommend them.

In the past several years there has been a trend in the treatment of emphysema toward using mechanical aids to respiration, especially in the form of intermittent positive pressure devices, and administering relatively low concentrations of oxygen in this manner. The hyperventilation so produced tends to "blow off" the excess carbon dioxide and, by improving ventilation, also helps to counteract the hypoxia. The improvement in arterial oxygen saturation may, however, be insufficient if excessively low oxygen concentrations are used. This feature is sometimes not sufficiently appreciated, as when patients with extreme hypoxia—who might better receive 50 to 60 per cent oxygen with the mechanical aid to respiration—are treated with compressed-air intermittent positive pressure breathing. The point was well made by Simpson (13) a few years ago that the ill effects of carbon dioxide narcosis may be reversible, but those of unrelieved anoxia are not. Careful observation for signs of narcosis in the patient under oxygen

therapy is, of course, essential whether or not intermittent positive pressure or artificial respirators are employed. The question of the circulatory effects of intermittent positive pressure devices must also be considered. A recent study at Oxford (14) of the mechanical properties of the chest during intermittent positive pressure respiration indicates that with some of these devices the intrathoracic pressure is permanently raised, because of the retarding of expiration, and this may significantly interfere with the venous return. If these devices are to be used, therefore, there must also be careful observation of the circulatory state, especially in patients with cor pulmonale and impending failure.

A particular caution to be observed, as has become well known in recent years, is the avoidance of sedatives such as morphine or barbiturates which may depress respiration and aggravate the effects of carbon dioxide narcosis.

Finally, the question of phlebotomy arises when the hematocrit is markedly elevated. Although the polycythemia, when present, may be regarded as a compensatory mechanism, there seems to be little doubt that the secondary effects are harmful. Small phlebotomies of 250 to 300 ml., repeated every few days to bring the hematocrit down to the level of 50, are usually recommended.

* * *

In closing, I should like to emphasize that there are many gaps in our knowledge and many points of disagreement among students of the pulmonary circulation. In summarizing the features which have seemed to me most important, I have tried to adhere to explanations of the pathogenesis and recommendations for the management of cor pulmonale about which there is fairly general agreement in the literature. Most of us who are called upon to treat these cases have not the opportunity or the technical training to carry out detailed physiologic studies of the type that have added so much to the understanding and the management of these conditions that may culminate in pulmonary heart disease. It is necessary to inform ourselves, however, and to apply in practice what the students of pathologic physiology have so brilliantly contributed by their painstaking and, often, conceptually very difficult studies.

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THE RESPIRATORY EFFECTS OF MEPERIDINE ALONE AND IN COMBINATION WITH LEVALLORPHAN IN PATIENTS WITH PULMONARY EMPHYSEMA[1]

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INTRODUCTION

Nalorphine and levallorphan, the allyl derivatives of morphine and levorphan, respectively, exhibit many of the pharmacologic effects of their parent drugs, but are singularly capable of antagonizing these and other narcotics when administered in proper dose. The effectiveness of narcotic antagonists in counteracting narcotic-induced respiratory depression in the human has been the subject of several investigations (1–8). With one exception (2), these studies have been interpreted as showing narcotic antagonists capable of preventing or alleviating respiratory depression when administered before, concurrently, or following a narcotic. They are ineffective against barbiturates and other sedatives, but counteract a variety of narcotics including those dissimilar in chemical structure.

Levallorphan has found wide clinical use in relation to anesthesia and postoperative care. It has been reported effective in preventing postoperative respiratory depression due to narcotics (4), in permitting larger doses of meperidine for its thiopental saving effect during anesthesia (9), and in reversing purposely induced narcotic apnea (6). An early report (10) suggested that nalorphine might be helpful in combating the respiratory depression of severe respiratory acidosis not related to narcotic administration; however, subsequent studies in normal subjects and in patients with respiratory acidosis demonstrated conclusively that nalorphine and levallorphan are in themselves capable of producing respiratory depression (1, 11). More recently, it was reported that a combination of levallorphan and meperidine in a ratio of 1:60 will prevent respiratory depression in postoperative patients that is induced by an equivalent amount of meperidine alone (12). No reduction in the analgesic potency of meperidine was found by using this ratio.

These reports, denoting the clinical value of levallorphan, suggested to the writers that a mixture of antagonist and narcotic might provide a means of safely administering potent narcotics to patients with severe respiratory insufficiency. It is well known that patients with advanced pulmonary insufficiency, particularly if it is accompanied by carbon dioxide retention, are sensitive to narcotics and prone to respiratory failure and uncompensated respiratory acidosis by their injudicious use. If the respiratory depressing action of a narcotic could be controlled without interfering with its analgesic effect, certain problems in management of these patients, such as pain relief and preoperative preparation, would be minimized.

In the present study the respiratory effects of meperidine and meperidine in combination with levallorphan were compared in each patient. Ratios of levallorphan to meperidine of 1:60 and 1:100 were tested, the latter proportion being that recommended by the manufacturer.[2] All patients who were evaluated, except for 3 without pulmonary disease, had obstructive pulmonary emphysema of moderate or marked severity; 5 of them had laboratory evidence of chronic carbon dioxide retention. No attempt was made to evaluate any but the respiratory effects of the drugs.

METHODS

Thirteen hospitalized male patients with pulmonary emphysema of moderate to severe degree and 3 patients without pulmonary disease were studied. All subjects were in the fasting state and had received no sedatives or narcotics in the preceding twelve hours. After a minimum of thirty minutes' rest in bed, an arterial puncture was made and the needle was fixed in the vessel. A mouthpiece attached to collecting tubing was then inserted. Fifteen minutes later, when the subject was judged to be relaxed, a three-minute sample of expired

[1] From the Medical Service, Veterans Administration Hospital; and the Albany Medical College, Albany, New York.

[2] Levallorphan is manufactured by Hoffmann-LaRoche, Inc. under the name Lorfan. A generous supply was kindly provided for use in this investigation.

air was collected in a Douglas bag. The respiratory rate was recorded and an arterial blood sample obtained during this period.

Following the control collections, either meperidine (1.5 mg. per kg. of body weight) or a mixture of levallorphan-meperidine was injected subcutaneously. Mixtures of levallorphan and meperidine in ratios of 1:60 (0.25 mg. per 1.5 mg.) or 1:100 (0.015 mg. per 1.5 mg.) were used. In 4 patients the mixture was given on the first day of the study; all others received meperidine initially. Thirty minutes after the injection, collections of expired air and arterial blood were repeated.

Each subject returned to the laboratory in three or four days for repeat studies. Resting measurements were made as previously described. If meperidine alone had been given on the first day of the study, the mixture was now administered, and vice versa. Expired air and arterial blood were again collected thirty minutes after the injection.

The volume of expired gas was measured in a Tissot apparatus. Samples of expired gas were analyzed for oxygen and carbon dioxide concentration, using a micro-Scholander apparatus. Arterial blood was analyzed for oxygen and carbon dioxide content by the method of Van Slyke and Neill (13). The oxygen capacity was measured according to the method of Roughton and associates (14). Blood pH was determined immediately at 37°C. with a Beckman pH meter. The carbon dioxide pressure (P_{CO_2}) was determined using the Henderson-Hasselbach equation. Physiologic dead space was calculated according to Bohr's equation substituting arterial P_{CO_2} for alveolar P_{CO_2}. Calculations of arterial oxygen saturation, mean tidal volume, and mean alveolar ventilation were made from these data.

Results

The ventilatory and arterial blood data obtained in each patient are recorded in tables 1 and 2. Individual increases in P_{CO_2} and decreases in alveolar ventilation are listed along the right margin of the tables, since they provide the most reliable evidence of the respiratory effects of the drugs. At first glance there appear to be discrepancies in the P_{CO_2} and alveolar ventilation values in several of the patients; however, when the percentage change is calculated for each variable, the findings are for the most part consistent.

Meperidine: Meperidine, administered to all patients in a dose of 1.5 mg. per kg. of body weight, resulted in an average increase of 7.1 mm. of mercury (17.1 per cent) P_{CO_2} and an average decrease in alveolar ventilation of 889 ml. (22.2 per cent). Only one patient (S.K.) of the 16 tested had ventilatory values comparable to control levels. Each of 3 subjects without pulmonary disease showed greater changes in P_{CO_2} and alveolar ventilation than the mean for the group. It is likely that this is fortuitous, resulting from the small number of patients tested, and should not be interpreted as evidence that patients with pulmonary emphysema react less profoundly to narcotics than others. The results are in agreement with the findings of other investigators (7, 12) that meperidine is capable of causing respiratory depression when used in therapeutic doses.

Since the subjects without pulmonary disease responded in essentially the same way as the patients with emphysema, the results for all patients are combined in subsequent tables and figures.

Levallorphan:meperidine 1:60: The comparative effects of meperidine and levallorphan:meperidine mixtures on P_{CO_2} and alveolar ventilation are illustrated in figure 1. The graph shows that the mean percentile increase in P_{CO_2} and the mean percentile decrease in alveolar ventilation produced by the 1:60 mixture exceeds that caused by meperidine alone. Individual analysis of this group (table 3) reveals that 3 patients showed a greater increase in P_{CO_2}, 3 had comparable increases, and 2 had less than that induced by meperidine alone. The results support the fact that levallorphan is a respiratory depressant and indicate that, when combined with meperidine in this ratio, it is not only ineffective but may actually potentiate meperidine respiratory depression.

Levallorphan:meperidine 1:100: The comparative effects on ventilation in patients receiving this ratio are shown in figure 1. In contrast to the group tested with the 1:60 mixture, these patients had less increase in P_{CO_2} and less decrease in alveolar ventilation relative to meperidine, showing a mean increase in P_{CO_2} of 9.8 per cent as compared with 14.8 per cent following meperidine. Nonetheless, as shown in table 3, 2 patients had greater P_{CO_2} increases with the mixture than with meperidine and 2 had comparable increases. Four patients responded with less increase in P_{CO_2}. It is noteworthy that only one patient (C.A.) had ventilatory values following the levallorphan-meperidine mixture equal to control values.

In this laboratory an increase in P_{CO_2} exceeding 5 mm. of mercury or a decrease in alveolar ventilation greater than 500 ml. is considered evidence of significant respiratory depression. An analysis of the data using these values (table 4)

TABLE 1

VENTILATION AND ARTERIAL BLOOD DATA
LEVALLORPHAN:MEPERIDINE, 1:60

Subject	Tidal Volume	Rate per Minute	Minute Ventilation	Physiologic Dead Space	Alveolar Ventilation*	O_2 Saturation	CO_2 Control	P_{CO_2}	pH	P_{CO_2} Increase	Alveolar Ventilation Decrease
	ml		*L*	*ml*	*L/min*	%	*vol %*	*mm Hg*		*mm Hg*	*ml*
K.W.†											
C	565	12	6.61	170	3.55	93.5	46.6	39.3	7.40		
M	503	12	5.89	159	2.94	93.6	49.5	46.5	7.35	7.2	610
C	1,033	11	11.69	348	6.8	94.0	46.5	31.7	7.50		
M + L	570	11	6.04	150	3.39	95.8	41.0	34.3	7.40	8.2	3,410
J.M.†											
C	631	12	7.38	193	4.05	99.3	47.1	38.3	7.40		
M	548	12	6.57	209	2.96	100.0	48.6	49.1	7.30	10.8	1,090
C	662	14	9.27	175	5.53	103.0	46.1	34.2	7.45		
M + L	471	13.6	6.27	150	3.05	100.0	49.1	45.0	7.35	10.8	2,480
R.C.											
C	538	12	6.45	186	3.12	91.8	54.4	45.8	7.40		
M	506	10	5.06	159	2.55	87.9	58.3	51.3	7.38	5.5	570
C	610	10	6.10	197	3.21	94.2	52.9	42.7	7.42		
M + L	489	10	4.89	188	2.09	87.9	57.2	53.3	7.35	10.6	1,120
E.D.											
C	607	25	15.17	276	5.98	98.7	43.9	29.4	7.50		
M	427	27	11.39	194	3.76	94.0	47.7	37.8	7.42	8.4	2,220
C	502	24	11.89	227	4.34	96.6	46.3	30.8	7.50		
M + L	361	24	8.77	146	2.99	93.1	44.6	40.7	7.42	9.9	1,350
C.Z.											
C	599	16	9.40	240	4.19	94.3	48.5	40.1	7.42		
M	577	13	7.33	222	3.34	91.9	52.3	46.8	7.38	6.7	850
C	764	13	10.16	340	4.42	96.1	48.9	41.6	7.40		
M + L	436	16	6.85	199	2.28	91.9	54.2	54.0	7.32	12.4	2,140
R.B.											
C	561	20	11.26	285	3.68	95.9	44.1	38.8	7.38		
M	559	18	10.07	289	3.20	94.9	45.8	45.2	7.32	6.4	480
C	565	19	10.90	286	3.61	95.8	45.2	37.8	7.40		
M + L	511	18	9.35	249	3.11	93.9	46.9	43.7	7.35	5.9	500
L.P.											
C	527	29	15.29	272	4.58	95.2	43.7	36.8	7.40		
M	492	25	12.15	274	3.12	92.7	45.6	46.0	7.31	9.2	1,460
C	523	29	15.24	275	4.63	94.0	43.3	35.2	7.42		
M + L	468	28	13.24	239	3.88	94.5	44.7	39.7	7.38	4.5	750
R.Cr											
C	655	15	9.83	224	5.09	91.5	50.0	44.0	7.38		
M	529	17	8.63	199	3.88	91.5	52.3	48.7	7.35	4.7	1,210
C	589	18	10.60	200	5.35	93.5	48.0	38.7	7.42		
M + L	573	16	9.16	209	4.38	90.3	50.4	47.4	7.35	8.7	970

C = Control
M = Meperidine
M + L = Meperidine + levallorphan

* An additional volume of 92 ml. representing apparatus dead space at BTPS was added to physiologic dead space volumes in calculations of alveolar ventilation.

† No pulmonary disease.

TABLE 2

VENTILATION AND ARTERIAL BLOOD DATA
LEVALLORPHAN:MEPERIDINE, 1:100

Subject	Tidal Volume	Rate per Minute	Minute Ventilation	Physiologic Dead Space	Alveolar Ventilation*	O_2 Saturation	CO_2 Control	P_{CO_2}	pH	P_{CO_2} Increase	Alveolar Ventilation Decrease
	ml		*L*	*ml*	*L/min*	%	*vol %*	*mm Hg*		*mm Hg*	*ml*
V.C.†											
C	524	12	6.13	110	3.77	93.6	47.5	40.0	7.40		
M	589	8	4.89	180	2.63	88.0	51.2	53.2	7.30	13.2	1,140
C	479	14	6.85	115	3.89	96.5	48.1	40.5	7.40		
M + L	563	12	6.76	156	3.78	95.5	50.6	44.6	7.38	4.1	110
C.A.											
C	485	18	9.69	251	2.84	94.7	61.2	52.5	7.38		
M	451	18	8.48	260	1.85	100.0	61.9	62.1	7.30	9.6	990
C	524	26	9.59	262	3.08	94.5	59.1	48.5	7.40		
M + L	466	25	8.39	226	2.66	100.0	60.3	49.1	7.40	0.6	420
W.L.											
C	447	26	11.61	226	3.35	82.3	51.5	42.6	7.42		
M	428	25	10.84	232	2.63	71.0	53.9	48.7	7.38	6.1	720
C	438	31	13.28	222	3.76	89.9	54.9	42.9	7.45		
M + L	—	—	—	—	—	80.9	59.6	49.7	7.42	6.8	—
L.S.											
C	626	16	10.02	273	4.18	86.9	60.1	51.5	7.40		
M	719	11	7.91	360	2.94	85.7	62.9	63.7	7.32	12.2	1,240
C	584	18	10.51	261	4.16	91.0	59.6	51.1	7.40		
M + L	699	11	7.69	289	3.50	94.2	62.3	55.8	7.38	4.7	660
H.B.											
C	378	21	7.94	159	2.67	95.0	55.3	49.1	7.38		
M	339	21	7.02	148	2.05	91.7	58.2	54.7	7.35	5.6	620
C	373	20	7.34	138	2.82	96.1	55.5	47.2	7.40		
M + L	366	19	6.85	151	2.30	92.9	57.5	51.1	7.38	3.9	520
S.K.											
C	479	16	7.81	185	3.29	88.6	51.7	53.7	7.30		
M	531	15	7.96	195	3.66	90.7	52.4	54.0	7.30	0.3	+370
C	465	24	11.17	193	4.32	94.3	50.6	47.6	7.35		
M + L	530	17	9.01	225	3.62	92.3	51.3	53.3	7.30	5.7	700
B.S.											
C	375	21	7.77	130	3.17	92.6	54.1	55.0	7.31		
M	368	16	5.89	121	2.48	83.5	58.9	60.4	7.31	5.4	690
C	400	21	8.53	158	3.20	86.1	55.9	56.1	7.32		
M + L	415	15	6.23	165	2.37	82.6	58.3	61.6	7.30	5.5	830
M.H.											
C	619	19	11.57	281	4.60	94.5	50.0	42.5	7.40		
M	554	15	8.31	203	3.89	89.2	53.6	45.6	7.40	3.1	710
C	622	24	15.12	278	6.12	93.2	46.3	38.3	7.42		
M + L	624	20	12.67	302	4.67	87.6	49.6	42.9	7.40	4.6	1,450

C = Control
M = Meperidine
M + L = Meperidine + levallorphan

* An additional volume of 92 ml. representing apparatus dead space at BTPS was added to physiologic dead space volumes in calculations of alveolar ventilation.

† No pulmonary disease.

provides an index of the potency of each of the drugs in producing respiratory depression. The results show that levallorphan-meperidine in a ratio of 1:60 produced a significant respiratory depression as frequently as meperidine, whereas the 1:100 mixture produced a significant rise in P_{CO_2} in fewer than half the patients. It is apparent that levallorphan when used with meperidine in a ratio of 1:100 offers protection to some patients against the full respiratory effects of meperidine. In others, however, no protective or even moderating effect can be demonstrated.

Because the respiratory center is depressed by prolonged exposure to increased pressures of carbon dioxide, the data were analyzed in an attempt to determine whether the respiratory effect of a narcotic is intensified in the presence of moderate chronic carbon dioxide retention. For the group, a resting control arterial carbon dioxide content in excess of 54 volumes per cent is considered evidence of prolonged carbon dioxide retention. Five patients of the 16 (R.C., C.A., L.S., H.B., and B.S.) had values in excess of this figure. Following meperidine the mean P_{CO_2} increase was 7.6 mm. of mercury (15.2 per cent) compared with an increase of 6.9 mm. of mercury (18.0 per cent) in patients without CO_2 retention. In this small group of patients carbon dioxide retention of moderate degree did not appear to influence the respiratory response to meperidine.

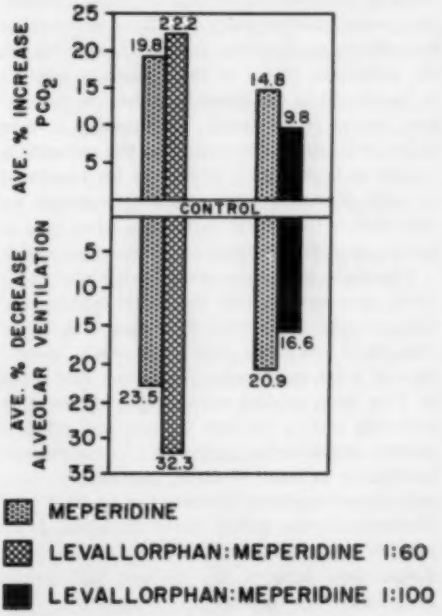

FIG. 1. Effects of meperidine and levallorphan:meperidine mixtures on arterial carbon dioxide pressure and alveolar ventilation.

TABLE 3

EFFECTS OF LEVALLORPHAN:MEPERIDINE MIXTURES ON P_{CO_2} INCREASE RELATIVE TO MEPERIDINE

Levallorphan + Meperidine	Number of Subjects	P_{CO_2} Greater	P_{CO_2} Less	P_{CO_2} Unchanged
1:60	8	3	2	3
1:100	8	2	4	2

TABLE 4

COMPARISON OF MEPERIDINE AND LEVALLORPHAN: MEPERIDINE MIXTURES IN PRODUCING SIGNIFICANT RESPIRATORY DEPRESSION

Drug	Number of Patients	>5 Mm. of Mercury Rise P_{CO_2}	>500 Cc. Fall of Alveolar Ventilation
Meperidine	8	7	7
1:60	8	7	7
Meperidine	8	6	7
1:100	8*	3	5

* Only 7 of this group had alveolar ventilation measured. One developed a leak in expired air sample.

DISCUSSION

Clinical observations have firmly established the effectiveness of narcotic antagonists in reversing respiratory depression produced by narcotic administration in the human. These compounds are now generally accepted as specific in the treatment of narcotic poisoning, and their value in alleviating *previously induced* narcotic depression is unquestioned. The evidence is less convincing that simultaneous administration of narcotic and antagonist or pretreatment with an antagonist will prevent narcotic respiratory depression.

In a study of patients lightly anesthetized with thiopental, Foldes and associates (8) found that a mixture of levallorphan:meperidine in a ratio of 1:66 caused no measurable reduction in ventilation from control levels. Similarly, pretreatment with levallorphan was found effective

in preventing meperidine respiratory depression. However, all patients had received 50 to 100 mg. of meperidine before anesthesia was induced and before control measurements were made, thereby introducing a modifying factor in the study. Furthermore, Pco_2 measurements were not made; changes in tidal volume, respiratory rate, and minute volume only were utilized in evaluating drug effect on respiration. It has been clearly shown by Loeschcke and co-workers (15) that measurements of total ventilation are relatively insensitive in detecting respiratory drug effect. They demonstrated diminished ventilatory responses to inhalation of carbon dioxide in subjects receiving narcotics (indicating reduced sensitivity of the respiratory center) in the presence of normal minute ventilation. Therefore, the effectiveness of levallorphan and other antagonists based exclusively on measurements of ventilation may be overestimated. The study of Megirian and White (12) has similar limitations.

Those investigations in which the ventilatory response to carbon dioxide was used to demonstrate a narcotic effect on the respiratory center offer contradictory evidence of the value of levallorphan in preventing respiratory depression. Shiffrin and associates (16) found levallorphan in a ratio of 1:100 to be completely effective in counteracting the respiratory effects of meperidine. Thomas and Tenney (1), using the narcotic levorphan in normal volunteers, found levallorphan in a 1:5 ratio effective in preserving the sensitivity of the respiratory center but ineffective in restoring its normal threshold to low concentrations of carbon dioxide, indicating incomplete antagonism. At the other extreme, Eckenhoff and co-workers (2) were unable to demonstrate any protective value of levallorphan when used with levorphan in ratios of 1:10 and 1:1 in normal subjects. They concluded that "the mixture of opiates and opiate antagonists for the purpose of preserving analgesia and preventing respiratory depression is not worthwhile."

Narcotic antagonists are in themselves capable of causing respiratory depression; therefore, an amount in excess of that needed to counteract the narcotic could produce ventilatory responses identical to a mixture containing inadequate antagonist. It would not necessarily be contradictory to find that ratios of 1:1 and 1:10 have no protective effect and that a 1:5 mixture has definite antagonizing properties. This may explain the results and the interpretations derived therefrom in the two studies mentioned above. The present study gives further evidence of the importance of the ratio between antagonist and narcotic: a ratio of 1:60 produced greater respiratory depression than meperidine alone, whereas a ratio of 1:100 produced less. Another reason for inconsistencies in reported results may be due to differences in time-dose relationships of narcotics and their antagonists on the respiratory center. In addition, there is probably considerable individual variation in the response to these drugs, so that a mixture of fixed proportions is unlikely to be effective in all patients.

It appears from the foregoing that narcotic antagonists are considerably more effective in alleviating existing narcotic depression than in preventing its development. The reasons for this difference are unclear since the specific mechanism of action of narcotic antagonists is not known. Thomas and Tenney (1), employing stimulus-response measurements, found that the depressant action of a narcotic and the antagonistic effect of levallorphan, as well, are most apparent when ventilation is under the drive of increased concentrations of carbon dioxide. In other words, the protective ability of the antagonist appears to be related to the stress to which the respiratory center is subjected. This finding is consistent with the observation that the antagonists appear to be relatively ineffective for treatment of mild depression (2), and may account for their failure to prevent depression when used in conjunction with therapeutic doses of narcotics.

The slight protective effect that it was possible to demonstrate with the 1:100 mixture was not sufficient to prevent significant Pco_2 elevation in 3 patients. Moreover, each patient showed a fall in alveolar ventilation and a rise in Pco_2 from control values. All patients were conscious during the test periods, and relief of anxiety undoubtedly contributed to the reduced ventilation in some of them, particularly those who hyperventilated during the control run. However, in the group tested with the 1:100 mixture, hyperventilation was not a significant factor since none of the patients had control Pco_2 values less than 38 mm. of mercury.

As far as clinical application is concerned, it makes little difference in these patients whether reduced ventilation is due partly to relief of

anxiety or to a direct action on the respiratory center. Suppression of ventilation of even a slight degree (irrespective of cause) may precipitate uncompensated respiratory acidosis in patients with severe emphysema, particularly if complicated by acute bronchopulmonary infection.

It is apparent from the results of this study that narcotic antagonists administered simultaneously with narcotic analgesics cannot be relied upon to prevent respiratory depression in patients with limited pulmonary reserve. The use of narcotics in this type of patient carries with it a risk of precipitating or aggravating respiratory acidosis whether the narcotics are administered alone or in combination with an antagonist.

Summary

The respiratory effects of meperidine and meperidine in combination with levallorphan were measured in 13 patients with pulmonary emphysema and in 3 patients without pulmonary disease. Levallorphan combined with meperidine in a ratio of 1:60 produced a greater mean increase in arterial carbon dioxide pressure and a greater mean decrease in alveolar ventilation than meperidine alone, whereas a ratio of 1:100 produced less.

Significant respiratory depression occurred in 7 of 8 patients tested with the 1:60 mixture and in 3 of 8 tested with the 1:100 mixture. In none of the patients did the ventilatory values following levallorphan and meperidine in combination equal the control values.

Levallorphan, when used with meperidine in a ratio of 1:100, provided incomplete protection against the respiratory effects of meperidine in some but not all patients. Levallorphan used in combination with a narcotic cannot be relied upon to prevent respiratory depression in patients with pulmonary insufficiency.

Sumario

Los Efectos Respiratorios de la Meperidina Sola y Combinada con el Levalorfán en los Enfermos de Enfisema Pulmonar

En 13 enfermos de enfisema pulmonar y en 3 enfermos sin afección pulmonar se midieron los efectos respiratorios de la meperidina sola y de la meperidina combinada con levalorfán. El levalorfán combinado con la meperidina en una proporción de 1:60 produjo mayor aumento medio de la presión arterial del bióxido de carbono (P_{CO_2}) y mayor disminución media de la ventilación pulmonar que la meperidina sola, en tanto que una proporción de 1:100 produjo menos efecto.

En 7 de 8 enfermos comprobados con la mezcla de 1:60 y en 3 de 8 comprobados con la mezcla de 1:100 hubo depresión respiratoria de importancia. En ninguno de los enfermos los valores ventiladores consecutivos al uso de la combinación de levalorfán y meperidina igualaron los valores testigos.

El levalorfán, cuando se usa con la meperidina en una proporción de 1:100, suministra protección incompleta contra los efectos respiratorios de la meperidina en algunos de los enfermos, pero no en todos. Usado en combinación con un narcótico, no puede confiarse en que el levalorfán impida la depresión respiratoria en los sujetos que padecen de insuficiencia pulmonar.

Resume

Les effets respiratoires de la mépéridine seule et associée au levallorphan chez des emphysémateux pulmonaires

Les effets respiratoires de la mépéridine seule et associée au levallorphan ont été mesurés chez 13 malades ayant de l'emphysème pulmonaire, et chez 3 sujets sans maladie pulmonaire. Le levallorphan associé à la mépéridine dans la proportion de 1 pour 60 produisit une augmentation moyenne plus importante de la pression artérielle de dioxyde de carbone (P_{CO_2}), et une diminution moyenne plus importante de la ventilation alvéolaire que la mépéridine seule, tandis qu'une proportion de 1 pour 100 produisit moins.

Une dépression respiratoire notable se produisit chez 7 des 8 malades soumis au mélange à 1 pour 60 et chez 3 des 8 malades soumis au mélange à 1 pour 100. Chez aucun malade, les valeurs de la ventilation, après levallorphan et mépéridine associés, ne furent égales aux valeurs de contrôle.

Le levallorphan, utilisé avec la mépéridine dans une proportion de 1 pour 100, produit une protection incomplète contre les effets respiratoires de la mépéridine chez certains malades mais pas chez tous. On ne peut pas se fier au levallorphan utilisé avec un narcotique pour prévenir une dépression respiratoire chez des malades ayant une insuffisance pulmonaire.

Acknowledgment

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PREHISTORIC TUBERCULOSIS IN AMERICA

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Introduction

This paper is concerned with the problem: Did tuberculosis exist in prehistoric America? By prehistoric is meant prior to the discovery of America by Christopher Columbus in 1492. Evidence of the existence of tuberculosis in the Old World for many centuries before Columbus is obtained to a certain extent from human remains (figure 1), but principally from numerous references in the Old World literature. These references include the description of the disease, diagnosis, cause, treatment, and prognosis (table 1).

Since there was no recognized written language in prehistoric America, evidence of the existence of pre-Columbian tuberculosis can come from two sources: art forms and skeletal remains. It seems advisable to discuss each of these somewhat in detail, but, for better understanding, it is important to know what the appearance would be of an art form whose live model had tuberculosis and what picture tuberculosis pathology leaves in specimens of dried bone.

Pathology of Bone Tuberculosis

Dr. J. Vernon Luck, in his book *Bone and Joint Diseases*, makes the following statement:

> The clinical diagnosis of skeletal tuberculosis is not a simple matter, and according to published statistics, A SUBSTANTIAL PERCENTAGE OF ERROR OCCURS EVEN IN THE BEST OF OUR ORTHOPEDIC CLINICS.

If one is confronted solely with a dried bone specimen, this error will certainly be multiplied many times. And furthermore, if one attempted to make a diagnosis from a prehistoric bone specimen, the only chance he would have of making even a good guess would be involvement of the spine. Tuberculosis in other locations would be indistinguishable from too many other diseases. It is true that tuberculosis involving the spine can cause different pathologic results, depending on virulence of the organism and resistance of the victim. If the host had little ability to defend himself, then tuberculosis could cause a rapidly progressive type of acute osteomyelitis, resulting in an early death. If the host's resistance was considerable, it is presumed that the disease then would be extra-chronic with the possibility of extensive healing and bone regeneration. Between these two extremes lies the pathologic picture of what is said to be typical spinal tuberculosis, without which it is impossible to make even a presumptive diagnosis of tuberculosis in a dried bone specimen.

The following are some of the characteristics of spinal tuberculosis:

(*1*) Tuberculosis of the spine usually involves one to four vertebrae. Involvement of more vertebrae does occur, but this is rare.

(*2*) Bone destruction occurs with little or no bone regeneration.

(*3*) As the disease advances, the bone in the vertebral bodies becomes eroded and decalcified. Under the pressure of body weight the spine collapses forward to give the characteristic deformity, the *angular kyphosis* (figure 2).

(*4*) Involvement of the neural arches and transverse and spinous processes is rare.

(*5*) Extravertebral "cold" abscesses are frequent. In the cervical and upper dorsal region these can occur posteriorly, and the sinus tracks can open externally. In the lower thoracic and lumbar areas, abscesses will develop anteriorly and occasionally rupture into the peritoneum or proceed to the psoas area, but they will almost never open through the skin posteriorly.

(*6*) Massive regeneration of the bone is a great rarity, and even spontaneous fusion is uncommon. That is why, before tuberculosis of the bone was treated with specific antituberculous drugs, so many cases necessitated surgical intervention.

The differential diagnosis of tuberculosis of the spine is shown in table 2. Any of the conditions listed could be confused with tuberculosis, especially if only the dried specimen was available.

Kyphosis: In the past there was a widespread

[1] Present address: Peoria Municipal Tuberculosis Sanitarium, P. O. Box No. 60, Peoria, Illinois.

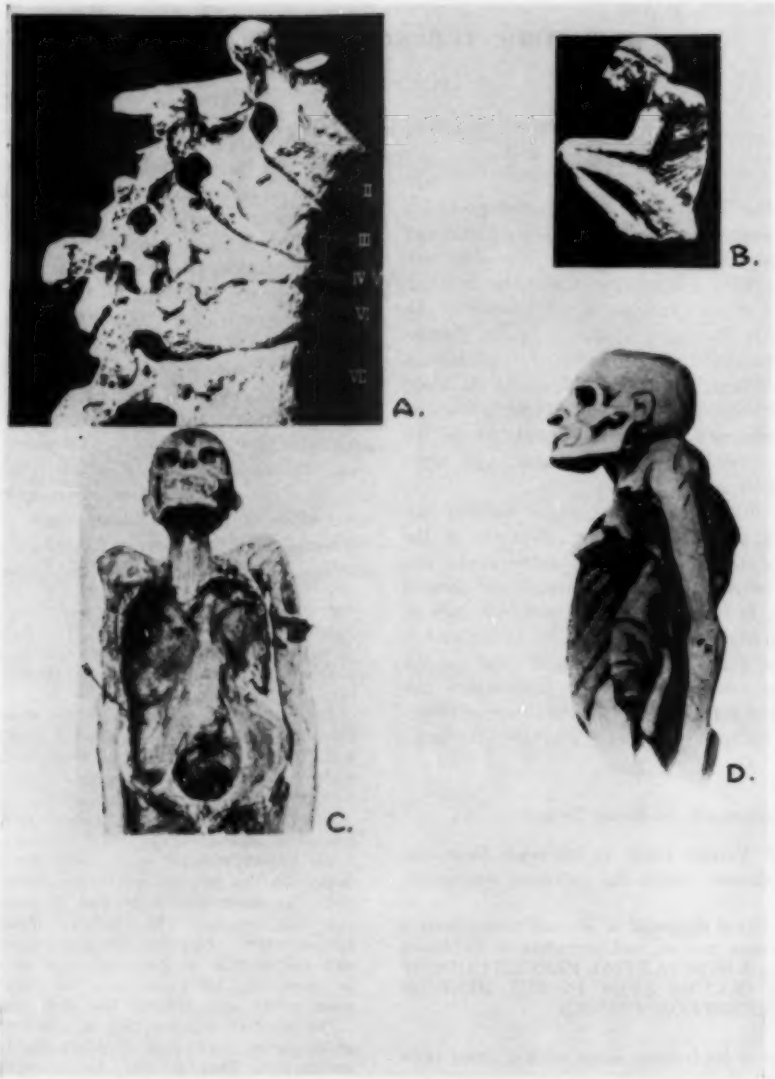

FIG. 1 *A*. Found in a Neolithic cemetery near Heidelberg in 1904 (5000 B.C.). Thought to be first evidence of human skeletal tuberculosis. Note involvement of the fourth and fifth thoracic vertebrae (from Paul Bartels); *B*. Egyptian clay statuette appearing to date at about 4000 B.C. Note angular kyphosis and emaciation (from Schrumpf-Pierron); *C*. Photograph of an Egyptian mummy after the front body wall was removed (XXIst Dynasty 1000 B.C.). There is partial destruction of the lower thoracic and upper lumbar vertebrae with a huge abscess (see arrow) occupying the right psoas muscle. Ruffer states in his report that other examples of tuberculosis have been found in Egypt in dynastic and probably predynastic times (from Ruffer); *D*. Artist drawing of *C* showing spine deformity (from Ruffer).

misconception that kyphosis was synonymous with spinal tuberculosis. The frequent references in literature that prehistoric hunchback art invariably means tuberculosis are not consistent with clinical facts. Besides the various diseases mentioned in the differential diagnosis table, developmental, nutritional, and endocrine disturbances are much more frequent causes of all sorts of spinal deformities, including kyphosis, than tuberculosis.

In Lincoln State School, located in Lincoln, Illinois, which is an institution for the mentally retarded, there are many severe deformities of the spine among the residents. Four of the most severe kyphotic cases which could resemble the models of prehistoric hunchback art were selected and are pictured in figure 3. Roentgenographic interpretation was made by Dr. Edward Wood, consulting radiologist at Lincoln State School. None of the roentgenograms gave the remotest indication that tuberculosis was the cause of the deformity.

Expected frequency: Assuming that tuberculosis did exist in a prehistoric American population and also assuming that treatment was either absent or at a very low minimum, what could we expect as to the incidence of bone tuberculosis in the skeletal material excavated. Some statistics are available on the tuberculosis morbidity and mortality of Indians in the United States during the early 1900's. At this time, treatment for tuberculosis among Indians was practically nonexistent, and most of the Indians lived in close contact with one another in overcrowded homes scattered throughout reservations. These conditions would be somewhat similar to concentrations of Indian populations in some parts of prehistoric America, such as our south central states, certain South American areas, and in the Southwest among the prehistoric Pueblo. The first statistics on this subject were by Dr. Aleš Hrdlicka who collected ninety-

TABLE 1

PRE-COLUMBIAN OLD WORLD LITERATURE

Hindu	
Vedas	?
Rig-Veda	2000 B.C. (?)
Yadur-Veda	400 A.D.
Manu	1300 B.C.
Hebrew	
Old Testament	700 B.C. (?)
Greek	
Homer	900 B.C.
Herodotus	450 B.C.
Hippocrates	400 B.C.
Isocrates	400 B.C.
Aristotle	350 B.C.
Roman	
Celsus	10 A.D.
Galen	175 A.D.
Aretalus	200 A.D.
Arabic	
Rhazes	900 A.D.
Avicenna	1000 A.D.
Middle Ages to 1492	
Medical writings and other literature contain many references to tuberculosis; evidence that tuberculosis was very prevalent, often occurring in epidemic form.	
King's evil	1050 A.D.

FIG. 2. Destruction of vertebral bodies resulting in the characteristic angular deformity of spinal tuberculosis (Girdlestone).

TABLE 2

DIFFERENTIAL DIAGNOSIS: TUBERCULOSIS OF SPINE

Chronic pyogenic osteomyelitis
Traumatic arthritis
Crush fractures
Malignancy
Typhoid spine
Sarcoidosis
Actinomycosis
Blastomycosis
Coccidioidomycosis
Rheumatoid arthritis
Osteitis deformans (Paget's disease)
Osteochondritis (Calve's—Scheuermann's)
Neuroarthropathies

FIG. 3. Spinal deformities not caused by tuberculosis. *A*. Wedging of vertebral bodies and marginal spurring; *B*. Compression deformity of thoracic vertebrae with no actual bone lesion; *C*. Fusion of several of vertebral bodies suggesting a congenital lack of segmentation; *D*. No significant bone changes.

one separate reports from physicians in the Indian service during the years 1904 and 1908, involving an Indian population of one hundred and seven thousand (table 3).

These reports noted that seven of every hundred cases of tuberculosis involved the bone. The eighty-first annual report of the Commissioner of Indian Affairs, published in 1912, recorded that, of a total population of one hundred and eighty-three thousand five hundred and eighty-seven Indians, there was a total of five thousand nine hundred and sixty deaths from all causes, and nineteen hundred and five deaths from tuberculosis. At that time, one of every three deaths among the Indians in the United States was caused by tuberculosis (rate 31.96). This would mean that in excavation of skeletal material, we could expect that, of every thousand deaths, three hundred and twenty were caused by tuberculosis. According to Hrdlicka, 7 per cent of all tuberculosis in living Indians involved the bone, which would suggest that 22.4 of every thousand burial specimens are going to show evidence of bone tuberculosis. Tuberculosis of the spine is the most frequent site of tuberculosis of the bone (more than 30 per cent); thus, of every thousand excavated skeletons there should be about 6.7 cases of tuberculosis of the spine. These statistics, of course, are not foolproof. Obviously, in 1904 and 1908 there must have been many cases of missed diagnoses, and the misses as far as pulmonary disease is concerned would probably be greater than the misses of bone tuberculosis. In addition, we are comparing Hrdlicka's incidence of 1907–1908 with the mortality figures in the Commissioner's report of 1912. Another factor that might alter this presumption would be that it is not known how much tuberculosis in the Indians of 1912 was caused by the human or bovine tubercle bacilli, as the incidence of bone involvement is thought by some to be highest when bovine bacilli are the cause. Regardless of the type of bacilli, bone involvement as a complication of pulmonary disease would be expected to be more prevalent when most of the cases were severe, advanced, and progressive.

Allowing for these statistical deficiencies, if prehistoric tuberculosis did exist in America, there should be many cases of typical spinal tuberculosis found among the large amount of excavated skeletal material.

TABLE 3

BUREAU AMERICAN ETHNOLOGY

(Vol. 42—Dr. Aleš Hrdlicka)

TUBERCULOSIS AMONG CERTAIN INDIAN TRIBES

(91 Reports 1904 and 1908)

Page 3

Indian population	107,000
Pulmonary tuberculosis	1,038
Lymph node tuberculosis	1,590
Bone tuberculosis	208

7 of every *100* total cases

20 bone cases to every *100* pulmonary cases

ART FORMS IN AMERICA

Dr. Gerald B. Webb in his book on the history of tuberculosis makes the following statement which has been repeated frequently in the literature:

> In pre-Columbian bronzes from Peru, in effigy water bottles in Indian burial grounds in many of our states, and from the pictographs of the cliff-dwellers, all pre-Columbian, the unmistakable picture of Potts disease is portrayed. This picture is so characteristic that orthopedic surgeons are certain that tuberculosis alone can be the cause.

Pre-Columbian bronzes from Peru: The Inca of ancient Peru manufactured many metal and ceramic effigies and figurines depicting various diseases and deformities. The exact number of authentic humpback figures from this area is unknown, but it is certain that they are very rare. Two are located in the U. S. National Museum (figure 4 *G*, *H*). The figure of the man on the llama (*H*) is 4½ inches tall and is made of silver. It came from Cuzco, Peru, and is pre-Spanish in time. The culture is Inca. Dr. Webb states that several of these bronzes are in the possession of the Army Medical Museum, and illustrates one of these in his book (figure 4 *I*). However, after correspondence with the Curator of the Museum and a personal visit, only one bronze figure was found to be on exhibit, and no information as to its authenticity could be obtained. The figure pictured in Webb's book could not be located.

Hunchback art can be found sparsely scattered throughout Mexico and Central and South America. Figure 4 *E* is a photograph taken of a dancer at Monte Alban, in southern Mexico (Zapotec culture). Other instances of figurines, vases, and pottery vessels depicting spinal deformities have been located in Venezuela, Guatemala, and Costa Rica. Many of these may be

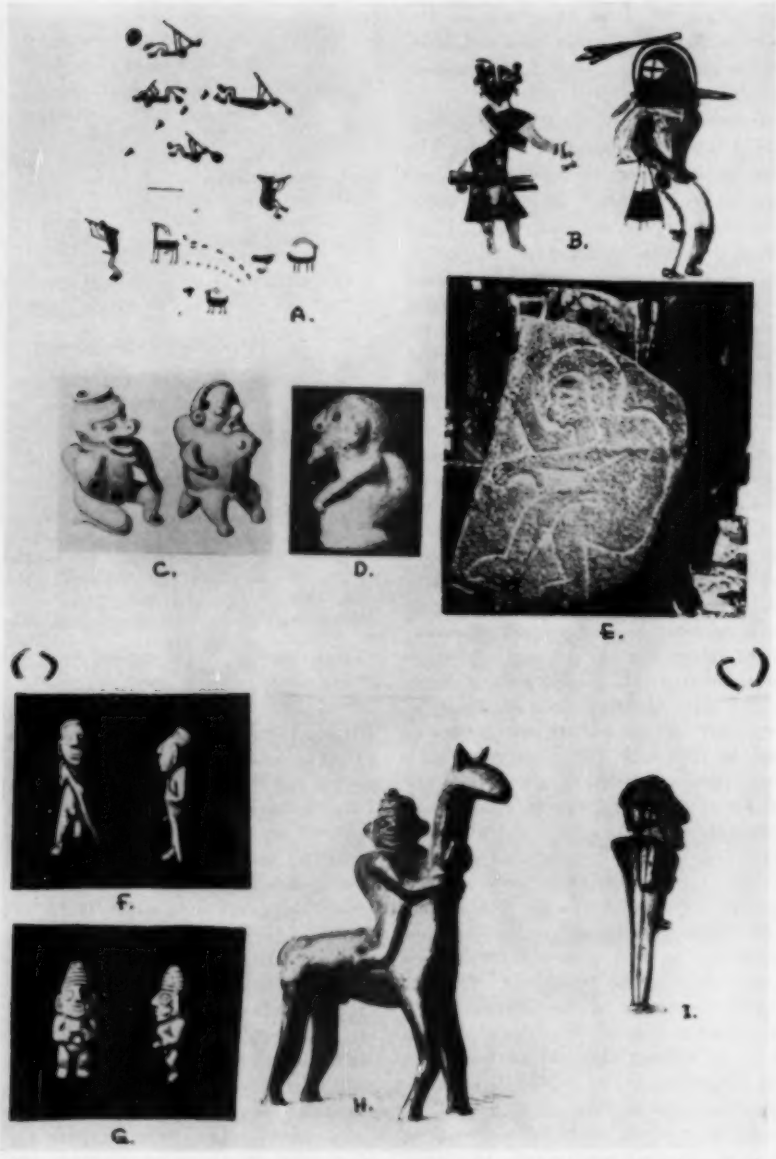

FIG. 4. Humpback art in America. *A*. Pictograph from Arizona showing humpbacked flute players. From Bulletin 65 B. A. E., Smithsonian Institution (*see* text); *B*. Female and male "Kokopolo" of the Hopi Katcina cult. The male is a humpback (from drawing in twenty-first annual report of the Bureau of Ethnology, Smithsonian Institution); *C*. Clay figurines from Costa Rica (from S. Linne, *Humpbacks in Ancient America*); *D*. Humpbacked ceramic figure from Venezuela (Height-5½ inches, from Requena); *E*. Humpbacked figure on stone block, Monte Alban, Mexico (Zapotec culture). Photograph by Dr. Dan Morse. One of the "dancers of Monte Alban"; *F*. Bronze figure of a cripple using a crutch,

historic. On some, it is doubtful that the intention was to portray deformity, but may represent a natural position of bending over, or the desire on the part of the artist to create symmetry. Others strongly suggest a pack on the back and not a deformed spine.

Effigy water bottles (figure 5)*:* In a small area of the central United States, including the corners of Arkansas, Tennessee, Mississippi, and Missouri, dozens of pottery bottles representing severely deformed hunchbacked human beings have been found by archaeologists. These water bottles have been identified as having been manufactured by people of a prehistoric Indian culture (late Mississippian). However, some recent dating strongly suggests that at least some of these may have been post-Columbian in time. The Banks Site, Crittenden County, Arkansas, where many of these vessels were found, gave a carbon date of 1535 A. D. Artifacts found elsewhere in this "Memphis" area suggest that other sites may also have a late date. It might be assumed that the original potters used human beings as their models for these vessels. The literature contains many references that these hunchback bottles are evidence of the existence of tuberculosis in the American Indian before contact with the white man. The writer has personally seen many of these bottles and viewed photographs of many others. The majority do not show the typical angular deformity of tuberculosis, but have smooth rounded curves with the prominent individual spines evenly spaced throughout the curvatures.

Pictographs in the Southwest: In figure 4 *A* a pictograph group is shown which was found near ruin 5 in Canyon Hagoe. Webb quoted these as representing spinal deformities due to tuberculosis with involvement of the cerebrospinal cord. Some of the group are pictured lying down, which is supposed to portray paralysis of the legs, and the playing on the flute is suggestive of occupational therapy. The figures which show priapism are supposed to be the result of spinal irritation. On the pictographs throughout the Southwest there have been many instances showing these hunchback flute players. From a medical standpoint, it is not likely that individuals with tuberculosis of the spine, in such an advanced stage as suggested in the pictures, would care much about lying on their backs playing flutes—they would be too busy dying. Elsie Parsons, in writing for *The American Anthropologist*, 1938, states that the humpback flute player displayed on the rock walls throughout the Southwest is not a human being, but represents an insect. This is the antecedent of the historic Hopi Kateina character called Kokopolo. The function of male Kokopolo (figure 4 *B*) is to chase women. Once he has seduced them he gives them a few presents which he carries in his hump. According to Dr. Frank Roberts in a study of the historic Zuni, it is believed that the humpbacked flute player may represent a rain priest, and that the frequently associated figures of horned toads and insects are symbols of magic. Furthermore, it is very likely that all of the pictographs in the Southwest made by prehistoric Indians were not meant to be accurate reproductions of certain objects, but have a legendary or mythologic meaning.

Skeletal Remains in Prehistoric America

The following fifteen cases of possible spinal tuberculosis are reviewed. Nine are taken from the literature, and six are reported here for the first time.

Case 1 (Putnam and Whitney). The first suggestion of tuberculosis in a prehistoric American Indian was from a published report by Dr. William F. Whitney of Harvard Medical School in 1886. Apparently, the Curator of the Peabody Museum, Dr. F. W. Putnam, asked Dr. Whitney to make a study of their osteologic collection. One skeleton,

from Cuzco, Peru. Received in 1901 by United States National Museum as part of Montez collection (Cat. No. 210368. Division of Archaeology). (*Photograph by courtesy of the Smithsonian Institution.*) The surface has been altered by smoking with ammonium chloride to bring out the details. This is an example of prehistoric treatment; the culture is Inca; *G*. Catalog No. 32265 (Division of Archeology) from Peru. Received in 1878 by the U. S. National Museum as part of the W. W. Evans collection. The surface has been artificially treated. (*Photograph by courtesy of the Smithsonian Institution*); *H*. Catalog No. 210366 (Division of Archeology) from Cuzco, Peru. Silver, 4½ inches tall. The culture is Inca and is pre-Spanish. Received by the U. S. National Museum in 1901. (*Photograph by courtesy of the Smithsonian Institution*); *I*. Bronze Peruvian figurine. From Webb's *History of Tuberculosis*. Said to be in possession of the Army Medical Museum, Washington, D. C. The Army Medical Museum was unable to locate or authenticate this statuette.

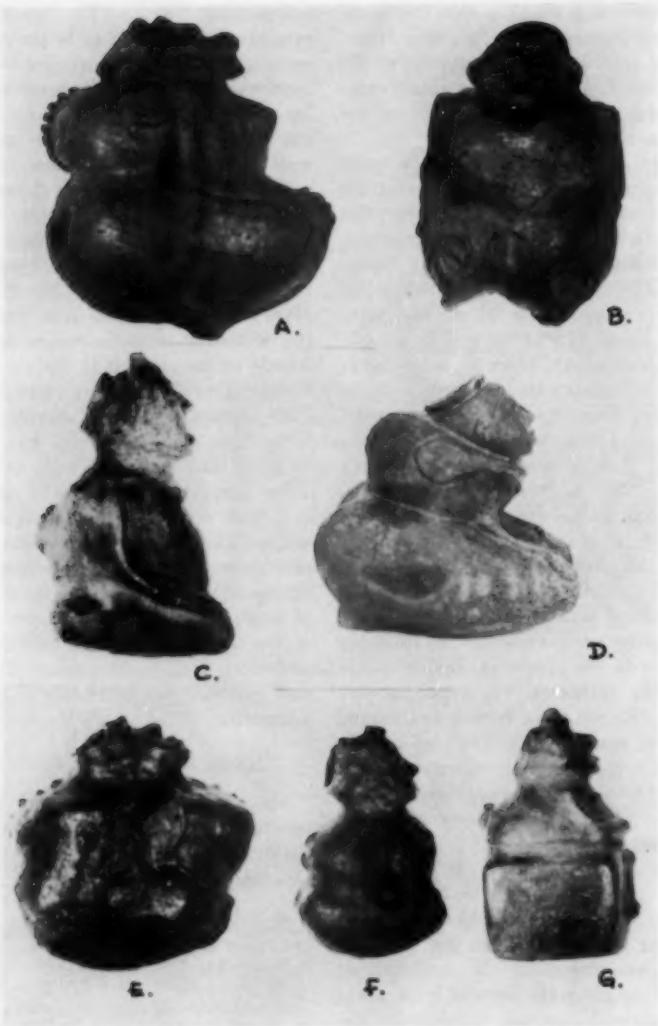

FIG. 5. Hunchback water bottles from the Memphis area. Mississippi culture (circa 1400–1600 A.D.). *A*. Obtained by Gregory Perino for the Gilcrease Foundation from the Banks Site, Crittenden County, Arkansas. It was found with Burial 70 and represents an old "crone" (toothless old woman); *B*. Front view of "A"; *C*. Found by Gray LaDassor of St. Louis, in Mississippi County, Missouri. Height is 7½ inches (Collection of Thomas Gilcrease Foundation, Tulsa, Oklahoma); *D*. Found February, 1959, at Bradley Site, Crittenden County, Arkansas, by T. W. Gitchell of West Memphis, Arkansas. The vessel is 6½ inches high. Note the smooth curve of the hump (Collection of Dr. Dan Morse); *E*. Obtained by Gregory Perino at the Banks Site. It accompanies Burial 244 with three other vessels. It represents a male with bulging chest. Note the five spinous processes. Height 6 inches (Gilcrease Collection); *F*. Found by LaDassor in Mississippi County, Missouri. It is 6 inches high (Gilcrease Collection); *G*. This vessel came from New Madrid County, Missouri. The knots on the back may represent a pack. The figure is seated on a "throne." Height is 7¾ inches (Gilcrease Collection).

catalogue No. 17223, from a stone grave mound near Nashville, Tennessee, showed an extreme case of anterior angular spinal curvature. "The disease had destroyed almost the whole of the bodies of the lower cervical or upper dorsal vertebrae and they had then become united in a firm mass."

Two other specimens, both from Tennessee stone graves, were reported by Dr. Whitney. One involved the knee joint and the other the ankle. He concluded that these two were very suggestive of bone tuberculosis, but his description of the specimens did not justify this conclusion.

Case 2 (Moore and Hrdlicka) (Figure 6*A*). Skeleton 277,730 is now located at the Department of Physical Anthropology, Smithsonian Institution, Washington. It was found by Clarence Moore in 1913 in a mound at Sorrel Bayou, Iberville Parish, Louisiana. The specimen was first examined and reported by Aleš Hrdlicka. The following description is by Dr. Hrdlicka: "The bones of skeleton 277,730 show considerable disease. They represent what is either tuberculosis or a very pronounced form of arthritis at the lower dorsal and especially the upper lumbar vertebrae, with moderate curvature forward of the spine." In addition Dr. Hrdlicka reports "an abscess cavity in the distal end of the left clavicle, more or less marked traces of periostitis of all the long bones, and signs of arthritis on one of the condyles of the right femur and lower articular surface at the right humerus." The bones represent one of the two skeletons in the mound which may belong to intrusive or later burials.

This specimen was seen and photographed by the present writer. All of the available vertebrae showed a moderate amount of osteoporosis and there was lipping of all bodies. Lumbar vertebrae 1, 2, 3, and 4 had a moth-eaten appearance on the surfaces of the bodies with numerous tiny bony nodulations (*see* photograph). The pathologic appearance of this specimen has no resemblance to tuberculosis.

Case 3 (Hooton). This case was reported by Dr. Earnest Albert Hooton in 1930, and the specimen was from the large amount of skeletal material excavated under the direction of Dr. A. L. Kidder from 1915 to 1924. It is now located in the Peabody Museum of Harvard. The specimen (catalogue No. 60,280) was a middle-aged female, forty-five to forty-nine, from Pecos Pueblo, New Mexico. It is thought to belong to Glaze IV time period which would date it about 1600 A. D. This would not only be post-Columbian but also post-Spanish. According to Hooton's Indians of Pecos Pueblo, the Spanish first visited the Pecos Village in 1540 under the leadership of Francisco Coronado. The specimen had a "degenerative arthritis deformans of the right shoulder joint and Pott's disease (?) or spondylitis deformans."

The present writer has not seen this specimen but from the photograph pictured in Hooton's book it appears that there is involvement of six vertebrae firmly fused together with only a slight deformity. The possibility is more likely that this was a severe arthritis than tuberculosis. Dr. Hooton states that in an examination of 503 Pecos Pueblo skeletons it was noted that 13.2 per cent had "spondylitis deformans" (arthritis of the spine).

Case 4 (Valcarcel and Garcia-Frias). In 1940, Dr. J. E. Garcia-Frias, Medical Director of the Clavegoya Sanitarium of Jauja, Peru, reported a case of what was thought to be tuberculosis in a Peruvian mummy. Mummies found in a certain arid area of Peru are dehydrated and actually contain flesh, skin, hair, and organs in a dried state, and by a process of hydration the tissues can be brought back to some resemblance of their original histology. This particular mummy was named Jorobado (meaning hunchback) and was supplied to Dr. Garcia-Frias by the National Museum of Archeology of Lima, Peru. The roentgenogram showed marked spinal kyphosis and compensating lordosis. From the ninth dorsal vertebra through the first lumbar, there was destruction of the bodies and fusion. Histologic studies of the hydrated lungs showed a large amount of fibrous tissue in the right apex. Dr. Garcia-Frias concluded that the combination of spine and lung disease showed that tuberculosis is the most likely diagnosis, and the present writer agrees, although other conditions are not completely ruled out.

The mummy Jorobado was pronounced to be prehistoric by Dr. Valcarcel, Department of Anthropology, University of San Marcos, Lima, Peru, and his co-workers from Cuzco. However, a pre-Columbian date is difficult to establish in certain instances because there is no distinct dividing line between pre- and post-Columbian. For a considerable time after the discovery of America, many Peruvian Indians had the same customs, used the same materials, and lived the same as their ancestors had for years before America was discovered by the white man.

Dr. Garcia-Frias reported on two other mummies studied by the hydration method: one had calcium deposits in hilar and mesenteric lymph nodes and the other had pleural adhesions. It is the opinion of the present writer that neither of the findings could justify a diagnosis of tuberculosis.

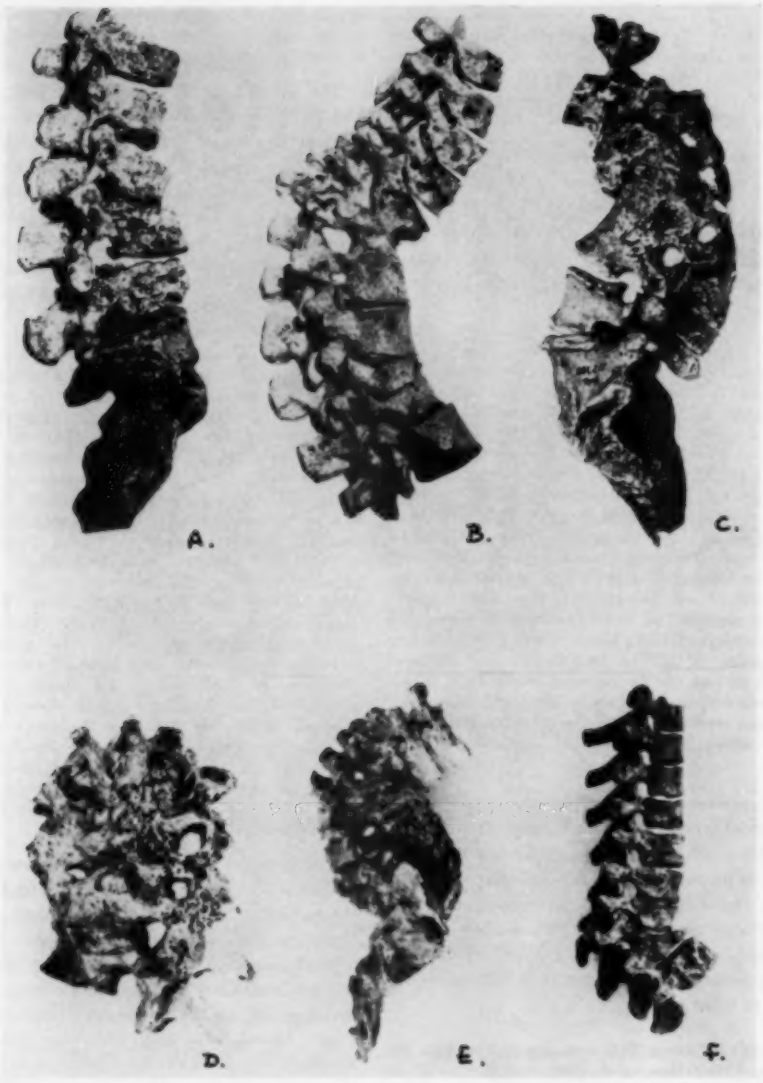

FIG. 6 *A. Case 2. (Use of photograph courtesy of Division of Physical Anthropology, U. S. National Museum, Catalog No. 277 730); B. Case 6. (Photograph by courtesy of Rochester Museum of Arts and Sciences and Dr. William A. Ritchie, Catalog No. AP 526); C. Case 9. (Use of photograph by courtesy of Division of Physical Anthropology, U. S. National Museum, Catalog No. 381 212); D. Case 13; E. Case 14; F. Case 15.*

Case 5 (Requena, Dupouy, and Cruxent). "The first unmistakable evidence of pre-Columbian tuberculosis in America" is a statement of Dr. Antonio Requena when describing a diseased human vertebra found in January, 1945, by Dr. Requena, Walter Dupouy, and Jose Maria Cruxent. There are two questions that should be answered in this case: Is the specimen pre-Columbian, and is the pathology typical of tuberculosis?

In a personal communication, Walter Dupouy kindly submitted a large amount of additional

information regarding the age of the specimen. The diseased vertebrae came from the El Palito archaeologic site, state of Carobobo, Venezuela. Although no carbon dating has yet been applied to the El Palito Site, other sites of the same age determined through comparison of styles and materials have been submitted to carbon 14 analysis. Dupouy refers to a monograph, *An Archaeological Chronology of Venezuela*, written by J. M. Cruxent and Irving Rouse. The archaeology of Venezuela was divided into five periods and the dates for each period were determined by various methods, including radiocarbon analysis.

According to the authors of that monograph, El Palito existed for a long span of time from late in Period II through Period III. Period II dated from 1050 B. C. to 350 A. D. and Period III from 350 A. D. to 1150 A.D. Any material found in El Palito would then date long before Columbus discovered Venezuela in 1498.

In regard to whether the specimen can be diagnosed as tuberculous, the following is taken from the writer's description: Almost all of the internal portions of the bodies of the tenth and eleventh thoracic vertebrae are completely destroyed, leaving a large cavity covered by a thin shell of bone. This cavity is connected to a large fistulous tract opening on the surface of the body of the tenth thoracic vertebra. A photograph included in the article shows the posterior view of the tenth and eleventh thoracic vertebrae. The caption under the photograph states: "The posterior surface, viewed from above, of the 10th and 11th dorsal vertebrae. We see on the posterior surface of the 10th, the exterior posterior opening of the fistula." Without seeing the specimen it is unfair to draw any conclusions but, according to the writer's description and the accompanying photograph, there are two features atypical of spinal tuberculosis: (*1*) failure of the collapse of the diseased vertebrae to create an angular deformity; and (*2*) the fistulous tract, described and pictured in the photograph, opening posteriorly, would occur very rarely in tuberculosis involving the lower thoracic vertebrae. Tuberculosis could be the cause of this pathology but it does not seem typical enough to justify the statement: "the first *unmistakable* evidence of pre-Columbian tuberculosis in America."

Cases 6 (figure 6 *B*), *7*, and *8* (Ritchie). In September, 1952, three cases of suspicious spinal tuberculosis from New York State were reported by Dr. William A. Ritchie. Each case came from a separate Indian culture. All three are in the possession of the Rochester Museum of Arts & Sciences. According to Dr. Ritchie, there is no doubt of the pre-Columbian origin of all three. The actual specimens or the photographs and the roentgenograms were submitted for opinions as to diagnosis to seventeen different medical specialists including pathologists, orthopedic surgeons, radiologists, and several physicians interested in prehistoric pathology.

The first specimen, catalogue No. A. P. 526, was excavated in 1938 in a cemetery of flexed burials, Livingston County, New York, by William Ritchie and Charles Wray. It was an adult female between twenty-six and thirty years of age. It belonged to the prehistoric Iroquois occupation and would date about 1200 A. D.

> "The lower seven thoracic and all lumbar, a total of 12 vertebrae, were involved in a destructive process. Necrosis of the bodies of the 10th, 11th, and 12th thoracic vertebrae resulted in approximately a 90° angular deformity. There is firm fusion of the lower thoracic vertebrae and another partial fusion of the 3rd and 4th lumbar. There are indications of multiple drainage canals involving the bodies and pedicles and articular facets and erosion of anterior body surfaces suggest a large perivertebral abscess enveloping the entire dorsal column."

Catalogue No. A. P. 529 was a skeleton of an adult male, forty years of age, found in Monroe County, New York, by Ritchie and Wray. It belonged to the Owasco culture which is thought to date somewhere between 500 and 1200 A. D. Ritchie describes this specimen as showing:

> a profound lesion which has destroyed the centra of the vertebral bodies of most of the thoracic segments of the spine, producing a kyphosis of slightly less than 90°. The remnants, together with the lumbar vertebral bodies, have undergone firm fusion. A marked atrophy of the anterior lumbar bodies may have resulted from the pressure of a thick walled-off abscess in the abdomen. Sinus formation and a slight amount of regenerated bone are also present.

Catalogue No. A. P. 530, the third specimen described by Ritchie, was found by relic hunters in 1938 in Seneca County, New York. The specimen consisted of seven ankylosed thoracic vertebrae. The rest of the skeleton was not saved. Ritchie checked the site and ascertained that it was pre-Columbian in time and belonged to the Middle Point Peninsula culture (date about 2,000 years ago).

> "The specimen consists of 7 thoracic verte-

brae, ankylosed into a solid piece which exhibits a complete kypho-scoliosis. The neural arch structures, both facets and laminae, are fused to the degree that they have completely lost their identity. The spinous and transverse processes are intact except for postmortem destruction, and the vertebral canal is patent throughout. Fine bony trabeculations extend throughout the fused bodies."

The medical specialists, in evaluating these three cases, were not unanimous in their opinions. The majority favored the diagnosis of tuberculosis as likely for all three but, as expected, there was considerable disagreement. The difficulties of diagnosing tuberculosis from the dried specimen alone are obvious. Not only is there a great scarcity of proved dried specimens of tuberculosis of the spine, but many cases of tuberculosis in the past, diagnosed principally roentgenographically, have never been verified. The one atypical finding common to all three of Ritchie's specimens is the extensive involvement (in A. P. 526, 12 vertebrae; in A. P. 529, possibly 17; and in A. P. 530, at least 7).

Case 9 (figure 6*C*). (O'Bannon and Lichtor). This specimen was discovered by Lloyd Gordon O'Bannon during a series of excavations undertaken by the Clarksdale Chapter of the Tennessee Archaeological Society in 1957. The skeleton came from Montgomery County, Tennessee, Site M. T. 17. It is from a stone box burial and belongs to the Mississippi culture which existed in this area, according to Professor Thomas M. N. Lewis, sometime from 1000 A.D. to 1600 A.D. The specimen is an adult; the sex could not definitely be determined. The description by Dr. Joseph Lichtor, orthopedic surgeon, is as follows:

> The specimen, the skull and spine of which have been accepted for inclusion in the National collections of the Smithsonian Institution, consist of a kyphotic thoracolumbar spine made up of seven vertebrae, the tenth thoracic through the fourth lumbar vertebrae. Except for the proximal one, all the intervening intervertebral discs have been destroyed. The vertebral bodies have collapsed and fusion has taken place. The intervertebral facet joints and neural arches are similarly affected. Throughout the kyphotic curve the spinal canal is patent and undistorted. The pathological changes are characteristic of spinal tuberculosis.

The involvement of seven vertebrae and the excessive bony regeneration are certainly not typical of the pathology caused by tuberculosis.

Case 10 (figure 7*C*). (Judd and Stewart). This is a child burial from Pueblo Bonito excavated by Neil Judd in 1947. The date is 828–1130 A.D. The specimen is now on display in an exhibit in the Armed Forces Medical Museum. There is partial destruction of bodies of T-12 and L-2, with almost complete destruction of L-1. There is fusion of the bodies of these three vertebrae. In addition there is a 45° angular deformity.

This case is quite typical of tuberculosis with destruction of vertebral bodies, angular deformity, and very little bone regeneration.

Case 11 (figure 7*D*). (Stewart). This specimen was excavated in 1955 at Nanjemoy Creek, Maryland, by Dr. Dale Stewart. The culture is Algonquin. The date is prior to 1608. No white contact material was found. All the lumbar vertebrae were involved and there are angulation deformities. Regeneration of bone is a prominent feature with complete fusion of L-1, 2, and 3. The specimen is on display at the Armed Forces Medical Museum, Washington D. C.

This case could be tuberculosis, but the amount of regeneration of bone is atypical. It also is probably not pre-Columbian in date.

Case 12 (figure 7*B*) (Nash). This case is located in the burial mound at Chucalissa, a State Park near Memphis, Tennessee. The burial is one of many excavated by the Parks Archaeologist, Charles Nash, and is permanently on exhibit. It is a male, age thirty to thirty-three, and belongs to the Mississippi culture. Five radio carbon dates have been obtained on this site, ranging from 1027 to 1617 A.D. This burial is in the upper part of the mound, which suggests that it probably belongs closer to the 1600 date. Although this is pre-white contact it is probably not pre-Columbian. Disease newly introduced could travel faster than the actual settlement of the territory by the whites.

This specimen comes very close to being typical tuberculosis. Two vertebrae are involved. The bodies of T-7 and 8 have apparently been completely destroyed and are not present. T-7 and 8 are fused at their inferior and superior articular processes, giving an acute angular deformity (about 45°). There is very little, if any, evidence of new bone formation.

Case 13 (figure 6*D*). (McGuirr and Dickson). In the early 1930's a farmer was digging for relics at Crables Indian Site, Kerton Township, Fulton County, Illinois. Knowing that Don Dickson was interested in bone pathology, anything the farmer found that appeared abnormal was brought to Dickson Mound Park. One of the specimens was a conglomeration of ten vertebrae, T-5 through L-1, all firmly fused together. The angle of deformity was about 160° (*see* photograph). The vertebrae were firmly fused through the bodies,

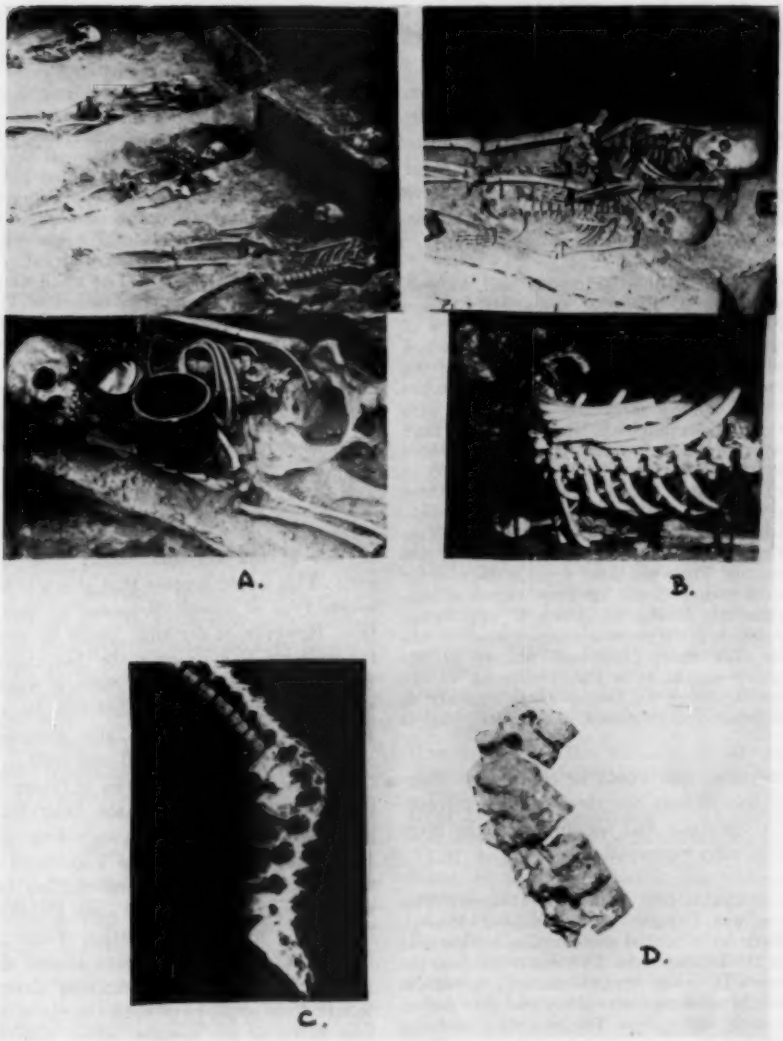

FIG. 7. *A. Case 14.* General view of Emmons cemetery with close-up of specimens *in situ*. *B.* Portion of burial mound at Chucalissa State Park near Memphis, Tennessee, with close-up view of deformed *Case 12 in situ*. (*Use of photograph by courtesy of Charles Nash, Tennessee State Parks Archaeologist*); *C. Case 10.* Roentgenogram of Pueblo child (*Photograph by courtesy of Army Medical Museum and Division of Physical Anthropology, U. S. National Museum*); *D. Case 11.* This specimen is on exhibit at the Army Medical Museum. (*Use of photograph by courtesy of the Division of Physical Anthropology, U. S. National Museum*).

neural arches, and transverse processes. There was tremendous regeneration of bone. The neural canal was patent. No other parts of this skeleton were saved, so it is impossible to tell the sex and age. However, the specimen appears to be from a small adult. Crables is a late Mississippi village site and cemetery. It appears to have been occupied by many people for a relatively short period of time. Three carbon-14 dates have been obtained by Dr. H. R. Crane, University of Michigan.

Specimens M-550, M-553, and M-554 were assigned the chronologic ages: 1338 A.D., 1358 A.D., and 1420 A.D.

The most prominent feature of this specimen is the extreme deformity and tremendous regeneration of bone. Involvement of ten vertebrae and massive bone regeneration would not favor a diagnosis of tuberculosis. Many burials have been excavated by individual diggers at Crables. No other similar specimens have been found.

Case 14 (figure 6*E*). (Emmons and Morse). This is burial 6, Emmons Cemetery, Fulton County, Illinois. It is a male, thirty-six years of age. The normal height should be 5 feet 6 inches, but, due to the deformity and telescoping of the spine, the individual would be about 6 to 8 inches shorter. The culture is middle Mississippi, with definite old village influence. No carbon dates have been reported as yet, but from the associated artifacts, the approximate date would be 1200 to 1300 A.D. All five lumbar and the lower three thoracic vertebrae are involved in a twisted compressed mass of bone destruction and massive regeneration. The fifth lumbar is pathologically fused to the sacrum. There are many holes in the anterior surfaces of bodies of the involved vertebrae suggesting multiple fistulas. There is a 45° angular deformity which probably was compensated by positioning. The most prominent feature besides the bony regeneration is the telescoping of the vertebrae. Although the neural arches are fused as are the transverse processes, the spinal canal is patent.

The features that would be contrary to diagnosis of tuberculosis are the extensive involvement (8 vertebrae and sacrum) and the large amount of bony regeneration.

Case 15 (figure 6*F*). (Hiles and Dickson). This specimen was brought in to Dickson Mounds State Park by a retired construction worker and given to Dr. Don Dickson. This was found near the surface in a Hopewell Mound but, since it was not scientifically excavated, its culture and date cannot be accurately determined. The reason for its being dug was that the mound was located on a hillside which had been optioned by a contractor who intended to use the ground for fill to repair a levee. No artifacts were found with this skeleton or with any of the other skeletons found buried near the surface. However, the two tombs located at the bottom of the mound contained Hopewell material, and many examples of late Woodland and Hopewell pottery were found scattered in the mound fill and in the immediate surrounding area. The writer's guess would be that the skeleton most likely belonged to a late Hopewell culture. Most of the skeleton was present. The skull was badly crushed. The left tibia, part of one fibula, an ulna, and most of the bones of the feet and hands were missing, as were the first cervical and the eleventh and twelfth thoracic vertebrae. It was the skeleton of a young male about twenty-five years of age, height 5 feet 3½ inches (possibly 2 inches less because of deformity). All thoracic and lumbar vertebrae except L-5 showed a moderate to severe amount of osteoarthritis as evidenced by lipping; bony spurs and a bony bridge joined L-3 and 4. There was a severe destructive osteomyelitis involving the bodies of thoracic vertebrae 5 through 10. Almost all of the body of T-8 was completely destroyed, with partial destruction of T-7 and about three quarters of T-9. Two small sinus openings were seen on the anterior surface of T-10. The neural canal was patent. Small nodules were seen on the surface of the bodies of T-5 through 11, indicating a moderate amount of bony regeneration with bone destruction, while there was a fusion of the bodies of T-8 and 9, involving a small part of the transverse processes. A 45° angular kyphosis was caused by the collapse of bodies of T-8 and 9.

With such an extensive destructive lesion, death must have occurred rather rapidly. Bony regeneration as seen by the nodules and fusion of two of the vertebrae must have occurred early. This would suggest that this was an acute severe type of osteomyelitis and of short duration. However, it certainly could be caused by tuberculosis. It is unfortunate that accurate excavation was not followed. Since it would seem that this individual was either late Hopewell or post-Hopewell Woodland, the probable date would fall between 200 A. D. and 1000 A. D.

Summary of cases: A series of fifteen cases of spinal disease occurring in the American Indian is presented. Of the fifteen only four (*Cases 4, 10, 12,* and *15*) could be considered typical enough to give a strong presumption of spinal tuberculosis. In one of these cases (Nash), a pre-Columbian date is not certain. Even in these four, other disease could have caused the same picture. In most of the remaining eleven cases, tuberculosis could have been the cause, but certain features are present which would throw some doubt on such a diagnosis.

Discussion

A contagious disease when introduced for the first time in any population is likely to be more severe because of a *lack of racial resistance*. This was true when many contagious diseases, such as measles, scarlet fever, and smallpox, were first introduced to the aborigines of America after the discovery of this continent by Columbus. For example, smallpox, according to Stearn and

Stearn, had a mortality rate in the colonial population of 12 to 14 per cent while, in the Indians, the mortality rate was 55 to 90 per cent. Untreated tuberculosis always seemed to be a more serious disease in the Indian than in the white man. Many medical authorities, however, who have had experience in tuberculosis control among the Indians believe that this apparent severity as measured by excessive mortality and morbidity rates is not due to any racial lack of resistance, but is caused wholly by unfavorable environmental influences.

Those who claim that tuberculosis did exist in prehistoric America offer evidence from two sources: prehistoric art and reports of spinal disease found in skeletal remains. The much quoted early American art forms, when carefully analyzed, add nothing to these claims. The fifteen cases of spinal disease reported here are not too impressive, since some were of doubtful pre-Columbian origin and only four could be considered typical enough to represent presumed cases of tuberculosis.

The subject, however, is not entirely settled. More evidence on either side may help to establish a final solution. This evidence may be forthcoming by further analysis of skeletal material yet to be excavated, or someone may come up with some sort of tuberculin test for dried bone. In the meantime: "Did tuberculosis exist in the prehistoric American Indian?" must be, at least temporarily, answered in the negative.

Acknowledgment

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PULMONARY AND CUTANEOUS NOCARDIOSIS[1]

The Significance of the Laboratory Isolation of Nocardia

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Introduction

Nocardiosis is a suppurative fungous disease usually involving the lungs and subcutaneous tissues. The diagnosis requires the demonstration by microscopy and culture of acid-fast, filamentous organisms which are aerobic. Some writers (1) have stated that the clinician must be wary of attaching significance to the laboratory isolation of nocardiae because of their saprophytic role in the respiratory tracts of patients with chronic bronchopulmonary disease. Others (2) have expressed the belief that nocardiae are frequent laboratory contaminants. Perhaps this latter view reflects the presence of Nocardia in the air (3) and in cultures of soil (4, 5).

Observations

If nocardiae were saprophytic for man, it should be possible to find them easily in a large series of carefully examined specimens. To test this theory, 235 specimens of sputum, gastric washings, and tonsils (removed at tonsillectomy) from 109 patients were examined by microscopy and culture. The subjects all had tonsillitis or other respiratory diseases, either tuberculous or nontuberculous. The result was that not a single isolation of Nocardia was obtained from these specimens (table 1). From this it is concluded that nocardiae are not frequent saprophytes of the human respiratory tract.

The ultimate diagnosis of nocardiosis was proved with bacteriologic methods. The widely held belief that nocardiae are frequent laboratory contaminants has often weakened the clinical diagnosis, as would any laboratory determination if it gave a high percentage of false positive values. The mycology laboratory of this study received 7,711 specimens for examination during the period from 1955 through 1959. At no time were nocardiae found that were not representative of active disease. In the tuberculosis bacteriology laboratory, the situation was only slightly different. During the five-year period, 44,071 specimens were cultured. Sodium hydroxide digestion and Löwenstein-Jensen medium were used. Seven cultures positive for Nocardia were found. This was an incidence of false positive cultures equal to 0.016 per cent. Of the seven positive cultures, four were from urine, two from sputum, and one from gastric washings. All had been submitted for examination for tubercle bacilli. None of the seven subjects had results positive for Nocardia on repeat culture using Löwenstein-Jensen and Sabouraud media. None of the subjects had nocardiosis, but three had pulmonary tuberculosis. From this it is concluded that nocardiae are rare laboratory contaminants.

Case Reports

Case 1. Pulmonary and cutaneous nocardiosis: L.J., a 54-year-old laborer, had cough, fever, and multiple draining subcutaneous abscesses which did not respond to tetracycline, erythromycin, or novobiocin. A 20-pound weight loss occurred during the two months of illness. His temperature was 104°F. and there was brawny induration of the right popliteal fossa. Fluctuant subcutaneous masses were present in the right lateral abdominal wall and the right antecubital fossa. Rales were heard over the right anterior chest. The chest film showed an infiltrate of the middle lobe with partial atelectasis. One of three sputum specimens was positive on microscopy and culture for Nocardia. The skin test with intermediate-strength PPD was negative.

Surgical drainage of the right antecubital fossa yielded 100 ml. of yellow cloudy fluid which contained branching, filamentous organisms that appeared gram-positive and acid-fast on microscopy. The culture was positive for Nocardia. Sulfadiazine in dosages of 6 to 8 gm. daily maintained the blood sulfonamide concentration at 13 to 14 mg. per 100 ml. The temperature returned to normal in two days, the middle lobe infiltrate resolved in three weeks, and the subcutaneous abscesses healed in three weeks. Sulfadiazine was administered for six months, and the patient has had no recurrence after three years.

[1] From the Veterans Administration Hospital; and the Department of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota.

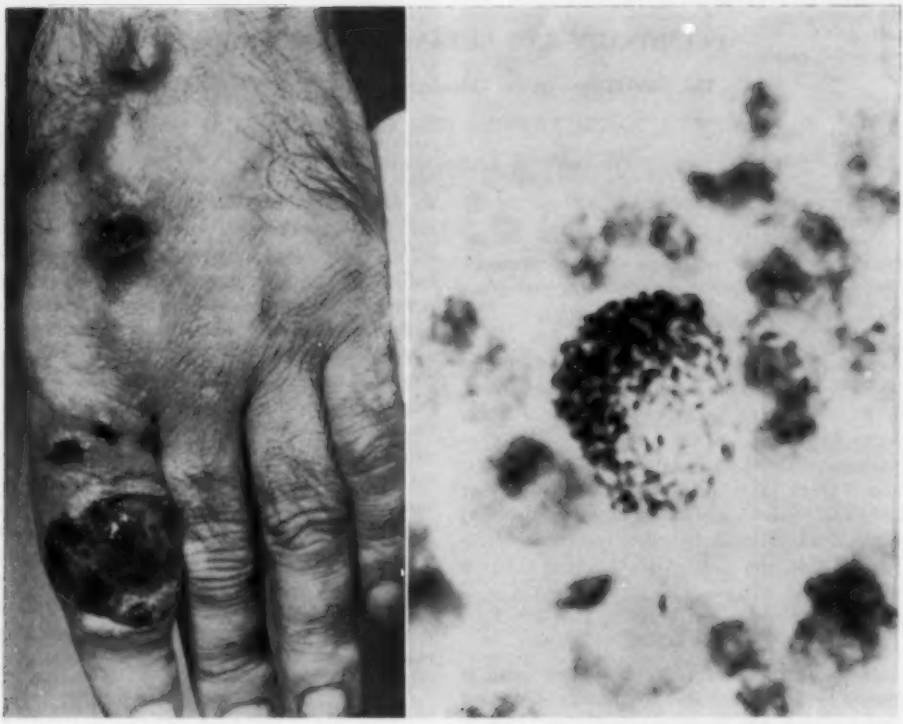

FIG. 1 (Left). *Case 2.* Cutaneous ulcers due to Nocardia; before sulfadiazine.
FIG. 2 (Right). Photomicrograph of exudate from ulcer showing unusual intracellular, acid-fast organisms *in globi.*

Case 2. Cutaneous nocardiosis and pulmonary sarcoidosis (6): B.D., a 38-year-old farmer, complained of swelling and suppuration of the left index finger for one month. Physical examination revealed a red, draining granuloma over the proximal interphalangeal joint of the left index finger, with firm subcutaneous nodules along the forearm (figure 1). Examination of the chest gave negative results. Microscopic examination of the exudate from the finger showed numerous intracellular, acid-fast organisms in bundles or "globi" (figure 2). Cultures for fungi at first were negative. A skin test with intermediate-strength PPD produced 12 mm. of induration. The chest film showed diffuse hazy infiltration of all lung fields.

Isoniazid and para-aminosalicylic acid were given for a presumptive diagnosis of tuberculosis; however, the finger lesion enlarged. Oxytetracycline, dihydrostreptomycin, and sulfone therapy produced no benefit. By this time, a culture of the exudate revealed Nocardia (figure 3), and 1 to 2 gm. of sulfadiazine were administered every four hours. The response was striking, with prompt regression of the skin lesions (figure 4). Four months of treatment were necessary before complete healing had occurred. The lung lesions were diagnosed as probable sarcoidosis because of a characteristic scalene node biopsy. The sputum was consistently negative for Nocardia. No recurrence of infection was evident three and one-half years later.

Case 3. Pulmonary alveolar proteinosis and pulmonary nocardiosis: L.T., a 40-year-old filling station attendant, complained of progressive dyspnea for six months. Cough with mucoid sputum had been present for two months. Three episodes of increased cough and thick sputum had responded to penicillin, but chest roentgenograms had not been obtained. Physical examination revealed this patient to be cyanotic and tachypneic, with bilateral basilar rales. There were no skin lesions. A chest roentgenogram showed extensive pulmonary infiltrates. A skin test with intermediate-strength PPD was negative. Sputum examinations for fungi were negative. A left-lung biopsy revealed pulmonary alveolar proteinosis; the alveolar spaces were filled with an eosinophilic granular material which contained acicular spaces.

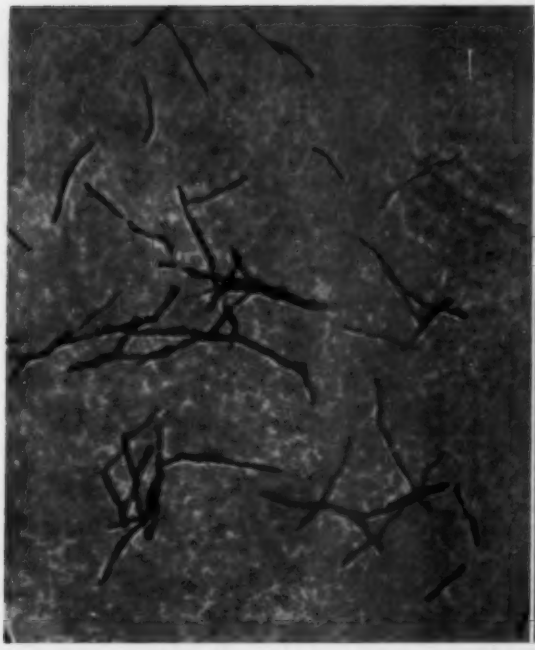

FIG. 3 (Left). Culture of Nocardia from ulcer showing branching, acid-fast filaments.
FIG. 4 (Right). Healing of cutaneous ulcers after sulfadiazine was given.

The patient was discharged with the understanding that he would continue prophylactic benzathine penicillin intramuscularly, but he soon returned with increased dyspnea and blood-streaked sputum. No response was gained from penicillin, chloramphenicol, erythromycin, or potassium iodide. On the day of death a tracheotomy was performed and two doses of corticotropin were given. A culture of sputum obtained seven days before death contained nocardiae. At autopsy the right upper lobe was found to be replaced by a large cavity lined with purulent and necrotic debris. Pus from this cavity was positive for Nocardia on microscopy and culture.

Case 4. Carcinoma of the lung, adrenal hypercorticism, and pulmonary nocardiosis: R.T., a 62-year-old machinist, complained of dyspnea, blood-streaked sputum, a 30-pound weight loss, and an ascending paresis for three months. Physical examination revealed generalized symmetric muscular weakness and atrophy. Rales were present in the left upper chest. A Guillain-Barré syndrome was verified; the cerebrospinal fluid contained 210 mg. per 100 ml. of protein and one leukocyte per high-power field. The chest film revealed a cavitating mass in the left upper lobe. The sputum was positive for Nocardia on microscopy and culture. A skin test with first-strength PPD was negative. The blood pH was 7.56, and the plasma corticoids were markedly elevated.

The patient was given sulfadiazine, 6 gm. per day; however, seven days later he became abruptly hypotensive and passed several tarry stools. Blood transfusions and pressor drugs did not help, and he soon died. At autopsy, two bleeding duodenal ulcers and several gastric ulcers were found. There was an adenocarcinoma of the left upper lobe bronchus. An adjacent 4-cm. cavity with a necrotic lining contained nocardiae. The brain, right lung, liver, spleen, kidneys, and adrenals contained metastatic adenocarcinoma. The zona fasciculata of the adrenals was hypertrophied.

Case 5. Pulmonary tuberculosis and nocardiosis: D.R., a 39-year-old pipe fitter, complained of dyspnea, cough with sputum, and night sweats for five months. Physical examination revealed a temperature of 103°F. and bilateral rales. Tenderness over the tibiae and fibulae suggested pulmonary osteoarthropathy, which was verified by roentgenography. The sputum was positive for acid-fast organisms on microscopy, and culture identified them to be nocardiae. The chest roentgenogram showed diffuse cavitary disease of the right upper lobe. A skin test with intermediate-strength PPD was negative.

Streptomycin and isoniazid were given for four

TABLE 1

LABORATORY SURVEY FOR NOCARDIAE

Source of Specimens	Number of Specimens	Nocardiae Found
Sputum (29 tuberculous and 22 nontuberculous patients)	153	0
Gastric washings (40 tuberculous and 11 nontuberculous patients)	64	0
Tonsils (all nontuberculous patients)	18	0
Total	235	0

weeks upon a presumptive diagnosis of tuberculosis, and resulted in no improvement. Sulfadiazine, 8 gm. per day, produced control of fever and cough and partial clearing of the lung densities after seven months. At this time the laboratory staff, noting *M. tuberculosis* in tubercles found in the organs of injected guinea pigs, suggested that pulmonary tuberculosis may also have been present. The patient meanwhile became ill again with cough and dyspnea. An infiltrate was now present in the left lung and the intermediate-strength PPD skin test produced 5 mm. of induration. Isoniazid and PAS were given, with some resolution of the pulmonary infiltrates and cavities. The sputum was negative for Nocardia, and no further sulfadiazine was given. At present the patient continues to receive antituberculous chemotherapy. Sputum specimens have become negative for tubercle bacilli.

Case 6. Pulmonary nocardiosis and Hodgkin's disease: M. F., a 42-year-old laborer, complained of anorexia and weakness. Following intermittent fever for twelve months, a scalene node biopsy had resulted in a diagnosis of Hodgkin's disease. Enlarged hilar nodes were seen on the chest roentgenogram. Nitrogen mustard controlled the fever, and adrenal corticoids were given for hemolytic anemia during the next twelve months. On admission, rales were present in the right midlung field. A chest film showed middle-lobe pneumonia.

The pneumonia did not respond to penicillin, chloramphenicol, or erythromycin. Four weeks later the entire right lung became involved. A right pleural effusion developed. Jaundice appeared and was attributed to liver involvement by the Hodgkin's disease. Sputum cultures were consistently overgrown with *Pseudomonas aeruginosa*. No endobronchial disease was seen on bronchoscopy. The patient became weaker and died three days later. After death the cultures of the bronchial washings were seen to contain Nocardia. The patient had received no sulfonamides. At autopsy both lungs contained nodules of Hodgkin's disease, and the right lower lobe was almost completely replaced by the tumor. A 5-cm. cavity with a necrotic lining was present within the tumor. Microscopic examination of the lining showed acid-fast, branching, filamentous organisms typical of Nocardia, but cultures were overgrown with *Ps. aeruginosa*.

DISCUSSION

The discovery of both pulmonary and subcutaneous abscesses often raises the first suspicion for a diagnosis of nocardiosis. No central nervous system involvement occurred in any of the present cases, although a review in 1957 of the reported cases of nocardiosis (7) showed a high incidence of brain abscess. The lack of response to chemotherapy other than sulfadiazine often gave a diagnostic lead.

The association with other lung diseases in the present series was striking. Pulmonary sarcoidosis, pulmonary alveolar proteinosis, carcinoma of the lung, pulmonary tuberculosis, and pulmonary Hodgkin's disease were present in the individual cases. Only one patient (*Case 1*) had nocardiosis alone. Larsen and associates (8), reported nocardiosis associated with Hodgkin's disease. In the twenty-seven cases of pulmonary alveolar proteinosis presented by Rosen and coworkers (9), two patients had nocardiosis. The frequent coexistence of nocardiosis with other lung diseases, usually chronic, has contributed to the erroneous opinion that nocardiae are frequently saprophytes of the human respiratory tract.

Nocardiae were readily isolated in this laboratory from specimens from patients in whom active disease was present. The acid-fastness was demonstrable in direct microscopic examination of purulent fluids, or from colonies on culture. Routine Ziehl-Neelsen decolorization methods (3 per cent hydrochloric acid and alcohol) demonstrated the organisms in all but one subject. In the latter patient, acid-fastness could be demonstrated only when decolorization was done with 1 per cent aqueous sulfuric acid. Culture was possible using Sabouraud and blood-agar medium, but pigmentation was more striking on Czapek agar. None formed cordlike colonies. Neutral-red binding was absent. Peroxidase activity was negative, but catalase activity was five plus. These tests were suitable for differentiating Nocardia from *Mycobacterium tuberculosis*, but the differentiation from the unclassified mycobacteria was more difficult. In this case, the rapid growth on many media and

the presence of branching filaments in cultures were the most reliable differentiating characteristics of Nocardia.[2]

Treatment of nocardiosis with sulfadiazine is now generally regarded as the best choice, although a recent review (1) suggested concomitant use of another antimicrobial drug. Of the present 6 infections, 3 responded well to sulfadiazine. The fourth patient received sulfadiazine for seven days, but died suddenly from a gastrointestinal hemorrhage. Specimens from the other 2 patients were negative on microscopy but positive on culture only a few days after death. No clinical response was shown to penicillin, tetracycline, oxytetracycline, erythromycin, chloramphenicol, novobiocin, streptomycin, isoniazid, and para-aminosalicylic acid. Blood sulfadiazine tests were utilized to maintain the concentration between 15 and 20 mg. per 100 ml. Strauss and associates (10) have demonstrated almost perfect protection with sulfadiazine alone in mice infected with Nocardia.

Summary

Six cases of pulmonary and subcutaneous nocardiosis are presented, treatment with sulfadiazine having been successful in 3 cases. Antimicrobial drugs and antituberculous chemotherapy resulted in no clinical response. Pulmonary sarcoidosis, pulmonary alveolar proteinosis, carcinoma of the lung, pulmonary tuberculosis, and pulmonary Hodgkin's disease were also present. Nocardiae were not found as saprophytes in the human respiratory tract and were rare as laboratory contaminants. When nocardiae were isolated, active disease was almost always present.

Sumario

Nocardiasis Pulmonar y Cutánea. Importancia del Aislamiento de la Nocardia en el Laboratorio

Preséntanse 6 casos de nocardiasis pulmonar y subcutánea, en 3 de los cuales había dado resultado el tratamiento con sulfadiacina. Los medicamentos antimicrobianos y la quimioterapia antituberculosa no provocaron respuesta clínica. También existían sarcoidosis pulmonar, proteinosis alvéolo-pulmonar, carcinoma del pulmón, tuberculosis pulmonar y enfermedad de Hodgkin pulmonar. No se encontraron nocardias como saprofitos en el aparato respiratorio humano y fueron raras como contaminantes de laboratorio. Cuando se aislaron nocardias, existía casi siempre enfermedad activa.

Resume

Nocardiose pulmonaire et cutanée. Signification de l'isolation de nocardia par le laboratoire

Las auteurs présentent six cas de nocardiose pulmonaire et sous-cutanée, traités avec succès par la sulfadiazine dans trois cas. Les médicaments antimicrobiens et la chimiothérapie antituberculeuse ne donnèrent aucune réponse clinique. Il y avait aussi des cas de sarcoïdose pulmonaire, de protéinose alvéolaire pulmonaire, de carcinome du poumon, de tuberculose pulmonaire et de maladie d'Hodgkin à localisation pulmonaire. Les nocardia ne furent pas trouvées comme saprophytes du système respiratoire humain et furent rarement des éléments de laboratoire contaminants. Quand on isola des nocardia, ce fut presque toujours dans des cas de maladie en activité.

[2] The organisms were classified as *Nocardia asteroides* because of the failure to liquefy gelatin or to coagulate milk.

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EXPERIMENTAL TUBERCULOSILICOSIS[1, 2]

A Comparison of the Effects Produced by Some of Its Various Pathogenic Components

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Introduction

The pioneer work on experimental tuberculopneumoconiosis in general and on tuberculosilicosis in particular has been done by Gardner (1), whose interest, however, was largely confined to the aggravational aspects of the dusts upon an existing or incipient tuberculosis. In an attempt to explain the mechanism of the disastrous alliance between silicosis and a tuberculous infection, Kettle (2) proposed the concept that tubercle bacilli have an affinity for silica; that they tend to localize and proliferate more readily in tissue containing quartz dust. Later work, however, has not supported this concept (3, 4). The most recent concept is one proposed by Vigliani and Pernis (5), which suggests that in silicotuberculosis there is a nonspecific reciprocal stimulation of immune responses.

The writers' interest in the mechanism of the synergistic relationship between quartz dust and tubercle bacilli was renewed when dusts other than quartz were demonstrated to have similar, although quantitatively less pronounced, effects upon experimental pulmonary tuberculosis (6, 7).

An investigation was begun, therefore, the purpose of which was to study the nature of the pulmonary disease produced by dead tubercle bacilli and by tuberculin in combination with quartz dust. An integral part of this investigation was a study of the pulmonary diseases produced by each of the components alone: dead tubercle bacilli, living tubercle bacilli, and tuberculin. These diseases were compared with those produced by quartz dust alone and by different combinations of the dust with one of the other components.

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Significant new aspects of a pulmonary tuberculin reaction have recently been reported as part of this investigation (8). Also, in another paper, a pulmonary disease resembling tuberculosilicosis has been described which is caused by quartz dust in combination with tuberculin (9). This disease is different in character and severity from that produced by either agent alone.

It is the purpose of the present report to describe and compare the pulmonary diseases produced by dead and living tubercle bacilli as well as by combinations of these organisms with quartz dust. In order to summarize the over-all results of the investigation, a tabulation is presented which includes the present, as well as the previously published findings.

Materials and Methods

More than 300 guinea pigs were rendered tuberculin positive by means of a subcutaneous injection of living tubercle bacilli which had a very low virulence for guinea pigs. This was the "B" strain used by Zaidi and associates (6) and was kindly sent to us by Professor C. V. Harrison of the Postgraduate School of London.

Following the demonstration of a positive tuberculin skin test on these animals, they were divided into groups of 30 or 15 and given intratracheal injections via the oral route by means of a laryngoscopic speculum (10). The experimental setup of the various groups and their injection schedule is given in table 1.

Animals that died prior to the first two months of the experiment were replaced from the stock of more than 30 tuberculin-positive guinea pigs held in reserve for this purpose.

Because the animals with single injections had received their dosage early, at a time when the technique of the intratracheal injections *per os* had not been perfected, some of these injections were apparently intra-esophageal since the lungs failed to show evidence of such injections. Undoubtedly, also, some of the animals scheduled to receive multiple injections did not receive their full intrapulmonary dosage for the same reason.

The quartz dust used in this investigation was incapable of producing silicotic nodules or collagen within the period of observation although the alpha quartz content by X-ray diffraction was

84 ± 6 per cent. Chemical analysis of this dust indicated an iron content of 8 to 9 per cent, and it is this iron which inhibits the development of silicotic nodulations and the formation of collagen (11). The average particle size of the dust was 0.7 micron.

In addition to the very early deaths which were replaced, there were later deaths due to pneumonia. Some animals were killed with ether at various intervals in order to study the progress of the various diseases. All surviving animals were killed with ether at the end of thirteen or fourteen months. Regardless of the manner of death, the lungs of all animals were inflated with formalin. Paraffin sections were cut from the inflated lungs and stained routinely with hematoxylin and eosin. Replicate sections from some of the animals were stained for acid-fast organisms.

Much information regarding the exact relationship of cellular tissue elements to their reticulin stroma was gained by photographing selected fields in routinely stained sections, decolorizing, impregnating with silver by the method of Gordon and Sweets, and then rephotographing the same field. Slides of quartz-containing tissue, after suitable photography, were incinerated at 600°C., treated with concentrated hydrochloric acid, and washed. The same fields were then rephotographed again but this time with dark-field illumination. Appropriate negatives were superimposed to give detailed information regarding the topographic distribution of the mineral as previously reported (12). A total of more than 4,500 negatives from slightly more than 1,600 fields was studied.

TABLE 1

EXPERIMENTAL SETUP OF INVESTIGATION

Group	Number of Guinea Pigs	Single or Multiple* Intratracheal Injections	Dose of Dust (total)	Dose of Other Agent (total)
			mg	*mg*
Quartz dust alone	15	S	50	—
Quartz dust alone	15	M	150	—
Living tubercle bacilli alone	30	S	—	†
Dead tubercle bacilli alone	15	S	—	1.0‡
Dead tubercle bacilli alone	15	M	—	3.0‡
Tuberculin alone	15	S	—	0.005§
Tuberculin alone	15	M	—	0.03§
Quartz dust plus tuberculin	30	S	50	0.005§
Quartz dust plus tuberculin	30	M	150	0.03§
Quartz dust plus living tubercle bacilli	30	S	50	†
Quartz dust plus dead tubercle bacilli	30	S	50	1.0‡
Quartz dust plus dead tubercle bacilli	30	M	75	3.0‡

S—single; M—multiple.

* The multiple intratracheal injections were given at monthly intervals. Some animals received a total of six.

† The dose was 1 ml. of a 10^{-2} dilution of a two-week-old culture of the "B" strain in Middlebrook-Dubos Tween® broth.

‡ These organisms were derived from the "B" strain. They were killed with concentrated formalin, thoroughly washed, and resuspended in saline. The dosage is calculated as dry weight.

§ PPD tuberculin.

RESULTS

Quartz dust plus living tubercle bacilli: The lungs of the animals which received quartz dust plus living tubercle bacilli were grossly deformed by bulging, gray-white, hard tissue which replaced much of the lung parenchyma in some lobes. Other lobes, however, showed little involvement. Fibrous adhesions to the hard gray tissue were common. Uninvolved portions of the lung were represented by depressed tan to brown regions. The lymph nodes were greatly enlarged and only moderately firm.

Microscopically, there was extensive consolidation of the lungs by large cells with ovoid vesicular nuclei, forming a tissue very similar to that observed in the lungs of animals reacting to the combination of quartz dust with dead tubercle bacilli or to the combination of quartz dust with tuberculin. There were, however, important points of difference. For instance, a much greater degree of collagenization was found in the lungs of animals reacting to the combination of quartz dust with living organisms than in the other groups. Also, foci of necrosis were more abundant and they tended to become confluent to form more or less continuous bandlike regions, often with narrow peripheral calcific borders.

The reticulin pattern was quite variable. In general, a fairly dense network of fibers had obliterated all air spaces, inclusive of bronchioles except near the periphery of the consolidation. In some zones of early necrosis the network of fibers was so dense as to justify the designation of sclerosis. In most zones of necrosis, there was an absence of all reticulin fibers in the center, whereas at the periphery, whether calcified or not, only the finer intra-alveolar fibers had disappeared so that the heavier fibers of the original septal walls stood

out like the columns of a ruined temple. There was accentuation of these necrotic septal walls by the silver impregnation (even though entirely indiscernible in the hematoxylin-eosin preparation) because of the collapse and condensation of the loose network of reticulin fibers. The reappearance of the original alveolated architecture was due essentially to a greater vulnerability of the finer intra-alveolar fibers to the lytic factors in the necrotizing process. The necrotic foci might contain irregular isolated fragments of a reticulin network. Such fragments might consist of short, thick, straight fibers or a small portion of a complicated network composed of interwoven and branching delicate fibers that gradually faded away at the periphery.

There were occasional noncircumscribed, small foci within some of the larger consolidated regions which were composed of loose aggregations of rounded macrophages with occasional to many giant cells. These cells usually contained little quartz dust, but many contained reticulin fiber fragments. In concordance with this latter evidence of destruction and phagocytosis of reticulin fibers, very little of the reticulin stroma remained in this region.

The quartz dust content of the involved lung tissue was highly variable and unpredictable. However, it was the rule to find little or no dust in regions of diffuse fibrosis at the periphery of consolidated regions where alveoli are lined by metaplastic epithelium. The latter gave such a region an adenomatoid appearance.

The satellite nodes showed a diffuse reticuloendothelial hyperplasia. In almost two-thirds of the animals there were also granulomatous foci, many of which showed central necrosis and some, calcification. The reticulin preparations exhibited three zones in the granulomatous foci, as have been described for similar foci in the lymph nodes of animals in the quartz-dust-plus-tuberculin group. These zones were an outer, circumferential zone of fibers; a middle, radial zone of fibers; and the inner necrotic zone.

Quartz dust plus dead tubercle bacilli: The gross involvement of the lungs of animals that received quartz dust plus dead tubercle bacilli approximated that of the preceding group but was more irregular. Some lobes showed no or very little consolidation. Other lobes had 25 to 90 per cent of their substance consolidated.

Microscopically, this consolidation was formed by the confluence of large nodular foci up to 1 to 2 mm. in diameter. Basically, the consolidation was an accretive type of chronic interstitial pneumonitis (13) with complete obliteration of all air spaces except for a few situated at the periphery of the component nodules. In about 90 per cent of the animals a variable number of the nodular foci showed central necrosis, and in more than one-third of the animals some of the necrotic regions were calcified. The calcified material had apparently dropped out in some of the foci so as to leave microcavities. In more than one-half of the animals there was a variable degree of collagen formation within the nodules. Multinucleated giant cells were found in remnants of air spaces, generally near the periphery of the nodules in some animals.

The reticulin preparation showed that, in or near the necrotic region, there was often an accentuation of the alveolar pattern, whereas elsewhere a network of fibers coursing in all directions had obliterated the air spaces. The accentuated outlines of the air spaces, as in necrotic foci of the preceding series, were produced by the dissolution and fragmentation of the fine intra-alveolar fibers while, at the same time, the multiple fiber layers of the thickened septal walls underwent condensation to form dense homogeneous bands of irregular contours. It was interesting to note that the necrotic foci in the lung never involved normal or near-normal tissue, but rather only the most severely altered granulomatous tissue.

A remarkable feature of this group was that the satellite lymph nodes of only 3 animals contained granulomatous nodules. This is the same incidence as was found in the silica-control group (9). However, these nodules were few in number, resembled those of the silica-control group, and were not as well defined or possessed of three zones, as those noted in the quartz-plus-tuberculin animals. The nodes were, in general, only moderately enlarged and this enlargement was caused by a hyperplasia of reticuloendothelial cells, some of which formed ill-defined clusters. In about one-fourth of the animals, multinucleated giant cells were associated with the proliferated reticuloendothelial cells.

The quartz dust content of the lung, as demonstrated by the incineration technique, was scanty in general but varied from region to region. Most frequently, the necrotic foci contained very little mineral dust, but exceptions

were noted. It was impressive to find large areas of alveolar obliteration without necrosis and without demonstrable quartz dust.

The quartz content demonstrable in the satellite lymph nodes was even less than that of the lung. Some of the giant cells and many of the reticuloendothelial cells contained dust, but others of the same type of cells did not.

Lungs and lymph nodes of animals dead three months or longer following the last intratracheal injection of tubercle bacilli, when examined for acid-fast organisms, were negative.

Living tubercle bacilli control: Grossly, the lungs of the animals that received living tubercle bacilli were normal. Microscopically, there were foci of accretive interstitial pneumonitis scattered throughout one or several lobes. In some animals, these foci were few and minimal in size. The lesion was characterized by a proliferation of alveolar cells which resulted in widening of septal walls to the point at which many air spaces were obliterated. This cell proliferation was accompanied by the development of a reticulin fiber network extending into the air spaces to a degree which was often less extensive than the cellular proliferation might suggest. Focal necrosis of lung tissue in regions of accretive interstitial pneumonitis was found in only one animal. This animal had died four and one-half months following the intratracheal injection of a spontaneous pneumonia which fortunately involved regions other than those of interstitial pneumonitis.

The satellite lymph nodes were only slightly enlarged, if at all. A reactive hyperplasia of reticuloendothelial cells was found in about one-half of the guinea pigs, and granulomatous foci were present in about one-third of these.

Dead tubercle bacilli control: The characteristic lesions in the animals that received dead tubercle bacilli were microscopic and consisted of nodular foci of consolidation, usually discrete. The consolidation was caused by an accretive type of chronic interstitial pneumonitis (13). Focal necrosis was present in the centers of some of the foci in about one-half of the animals. In about one-half of the animals which exhibited focal pulmonary necrosis, there was also calcification of the necrotic tissue. Some of the necrotic calcified material had dropped out so as to leave irregular microcavities in a few animals.

The essential difference in the character of the lesion in this series of animals as compared with the lesion in the series given dust in addition to the dead bacteria was the discreteness of the nodules in the former and their confluence to produce extensive, almost lobar consolidation in the latter.

The satellite lymph nodes were not enlarged in more than one-half of the animals. Microscopically, the nodes of about three-fourths of the animals showed no significant abnormality other than edema. The lymph nodes of the other animals exhibited a reactive hyperplasia. No suggestion of a granulomatous inflammation was found.

Acid-fast stains revealed no organisms after a time lapse of three months or longer following the last intratracheal injection of dead tubercle bacilli.

Other animal series: The basic lesion of the other three series of animals, as is true for the four series herein described, was an accretive type of chronic interstitial pneumonitis (8, 9, 13). The incidence, severity, and extent of this lesion varied with the agents and dosage introduced into the lungs as well as with other factors. The satellite lymph node involvement was similarly a function of these agents, their dosage, as well as other factors which will be discussed later.

The incidence of animals with the most significant lesions produced by the various pathogenic agents is given in the summary of table 2.

Discussion

The characteristic lesion of experimental tuberculopneumoconiosis is an extensive, fibrous, obliterative alveolitis. This obliteration of air spaces may be associated with focal necrosis with or without calcification and with or without cavitation (6, 7). The pulmonary inflammatory response to the combination of nonfibrogenic quartz dust with dead tubercle bacilli possesses all of the above features.

It would appear that the variability in the severity of the disease produced, i.e., the degree of collagenization, the incidence and severity of the necrosis, calcification, and cavitation, are dependent upon the reactivity of the host, the virulence and dosage of the living or dead bacteria (as the case may be), as well as the character and amount of the dust.

A smaller dosage of dead tubercle bacilli might have been expected to have resulted in a reduction of the incidence and extent of the

TABLE 2

INCIDENCE OF GUINEA PIGS WITH LESIONS OF LUNGS AND LYMPH NODES AS A RESULT OF DIFFERENT INTRAPULMONARY PATHOGENIC AGENTS

Intrapulmonary Pathogenic Agent	Pulmonary Lesions				Lymph Nodal Lesions			
	Interstitial Pneumonitis	Collagen Formation	Focal Necrosis	Focal Calcification	Reactive Hyperplasia	Granulomatous Foci	Focal Necrosis	Focal Calcification
Quartz dust alone	++++	±	±	−	++++	+	±	−
Living tubercle bacilli alone	++++	−	±	−	++	+	−	−
Dead tubercle bacilli alone	++++	+++	+++	++	+	−	−	−
Tuberculin alone	+++	−	−	−	+	−	−	−
Quartz dust with living tubercle bacilli	++++	++	+++	+	++++	+++	++	+
Quartz dust with dead tubercle bacilli	++++	+++	++++	++	++++	+	±	−
Quartz dust with tuberculin	+++	+	+	−	++	++	++	−

− None
± up to 10 per cent
+ about 25 per cent
++ about 50 per cent
+++ about 75 per cent
++++ about 100 per cent

focal necrosis, calcification, cavitation, and collagenization. A substitution of pure quartz dust for the iron-contaminated quartz dust would, in all probability, have resulted in an increased incidence of collagenization as well as of necrosis and calcification of the pulmonary parenchyma.

The fact that a nonfibrogenic quartz dust in conjunction with either tuberculin (9) or dead tubercle bacilli can simulate tuberculosilicosis suggests that the latter disease is the result of a potentiated inflammatory response to the dust by the tuberculous infection, and vice versa, as has been postulated by Vigliani and Pernis (5).

One of the interesting differences between the diseases caused by dead and living tubercle bacilli, respectively, whether with or without quartz dust, is the failure of the dead organisms to cause granulomatous lesions in the satellite nodes. Those few lesions found in the quartz-dust-plus-dead-tubercle-bacilli group (none were observed in the group injected with dead organisms without the dust) must be ascribed to the action of the quartz dust inasmuch as the anatomy of the lesions and their incidence was exactly the same as those observed in the dust-control group.

The conclusion which seems to be forced is that the number of dead tubercle bacilli transported from the lungs to the satellite nodes was too small to produce lesions even in the presence of quartz dust. It is possible that the number of living organisms transported to the lymph nodes was no greater than the number of dead organisms so translocated but, because of the greater pathogenic potential of the former and their ability to multiply, the production of lymph node lesions was facilitated.

As indicated in a previous paper (9), the high incidence of granulomatous nodules in the quartz-dust-plus-tuberculin group suggests that some of the injected tuberculin was not fixed by the lung tissue. Enough uncombined tuberculin apparently reached the satellite nodes to act synergistically with the quartz dust and produce the characteristic nodules.

The pulmonary response to an intratracheal injection of dust combined with dead tubercle bacilli had been studied by Kettle (14), Cummins (15), and (relatively recently) by Attygalle, Harrison, King, and Mohanty (16).

The common finding in all of these studies has been that the inflammatory response produced by the combination of dust with dead tubercle bacilli was greater than the sum of the reactions produced by each agent acting alone.

Furthermore, Attygalle and associates (16) noted that the inflammatory response to the combination of dust with dead tubercle bacilli was one which tended to regress within one year

in guinea pigs. However, in the writers' experiments, no regression was observed in the inflammatory response to the combination of dust and dead tubercle bacilli.

This difference in results need not be disturbing, nor should it be considered contradictory, since nearly every essential factor was different in the two investigations. The factors referred to are:

(*1*) The reactivity of the host. The writers used tuberculin-positive guinea pigs as against nonsensitized guinea pigs used by Attygalle and co-workers (16).

(*2*) The pathogenicity and dosage of the dead organisms. The writers used up to 6 mg. (dry weight) of formalin-killed human tubercle bacilli, Harrison's "B" strain, as against a maximum of 4 mg. (moist (?) weight) of heat-killed BCG used by the British investigators.

(*3*) The character and dosage of the dust. The writers used a dust containing about 84 per cent quartz as compared with dusts used by Attygalle and co-workers which contained only up to 6 per cent quartz.

The importance of the pathogenicity and dosage of the dead tubercle bacilli injected is underscored by the vast difference in the character of the lesions produced in the control animals as compared to that of the control animals in Kettle's (14) experiment as well as that of Attygalle and co-workers (16).

Kettle (14), for example, found no necrosis or fibrosis following the intratracheal injection of dead tubercle bacilli (dosage and origin or virulence of organisms not given). He stated, "... I have found nothing which suggests that dead tubercle bacilli are capable of producing permanent lesions when introduced into the lungs of guinea pigs by intratracheal injection." The more recent results of Attygalle and associates (16) with dead BCG were similar to those of Kettle since the former noted that BCG caused only a mild reticulinosis.

This suggests that the present writers had unwittingly used a much larger dosage and probably also a more pathogenic organism than had the other investigators since, in the animals in these experiments, the dead tubercle bacilli produced not only collagenous fibrosis but also necrosis and calcification. Also, the considerably greater pathologic effect of the dead tubercle bacilli compared with that of the living tubercle bacilli is another indication of a relatively excessive dosage of the former. It would appear that there might have been a much greater difference in the severity of the lesions caused by dust plus dead organism than in those caused by dead organisms alone had the dosage of the latter been less than that used.

Although the basic inflammatory response to the combination of quartz dust with living tubercle bacilli is very similar to the inflammatory response to the same dust combined with dead tubercle bacilli, the latter response differs in the following respects from the former: The necrotic foci are discrete and limited to the center of the confluent nodules rather than interconnected and bandlike: calcification of the necrotic material may be extensive rather than limited to a thin marginal zone; there is more collagen formation; the granulomatous involvement of satellite nodes occurs in only a few animals and is minimal in degree, in contrast to the high incidence of such lesions in animals injected with dust plus living organisms. Similarly, the quartz content of satellite nodes appears to be less in animals injected with dust plus dead tubercle bacilli than in animals of the group injected with dust plus living tubercle bacilli.

The status of the reticulin fibers in relation to focal necrosis was of considerable interest. The absence of reticulin fibers in some necrotic foci was understandable, but it was perplexing to note the presence of accentuated, broad, and dense reticulin structures representing septal walls within such foci. This was even more remarkable when the same area stained with hematoxylin and eosin gave no indication that these structures were present. From a study of the reticulin fibers at the margins of necrotic zones it was concluded that the necrosis occurred only in regions of maximal obliterative inflammatory change and that the perifocal inflammatory changes were pre-existent to the necrosis. If the findings and conclusions based on these experimental lesions are applicable to those encountered clinically, then the concept that a zone of perifocal inflammation is a reaction to the presence of a necrotic focus may require revision.

The ability of a soluble metabolic product of tubercle bacilli as well as of their lifeless bodies to produce a tuberculopneumoconiosis-like disease in conjunction with dust raises the question to what extent, if any, does this ability become

a practical consideration in the evaluation of clinical disease? For example, this question may arise in the so-called progressive massive fibrosis of the coal-miner's lungs which is considered by British investigators to be a tuberculoanthracosis. In this disease, tubercle bacilli are demonstrable by culture or animal inoculation in only about 40 per cent of the autopsied cases (17). In the lungs of some of the patients in whom no tubercle bacilli are demonstrable, may there be depots of tuberculin unfixed or only loosely fixed to antibodies or tissue? Such tuberculin, if present, could act synergistically with dust as the intratracheally injected tuberculin has been demonstrated to act. In view of the persistence of the experimental pulmonary tuberculin reaction for many months after an intratracheal injection of tuberculin (8), the survival period of reactive tuberculin in living tissue should be an interesting project for future investigation.

Summary

Tuberculin-positive guinea pigs were divided into groups and were injected intratracheally with one of the following pathogenic agents or combinations of agents: iron-coated quartz dust, living tubercle bacilli, formalin-killed tubercle bacilli, tuberculin, iron-coated quartz dust plus living tubercle bacilli, iron-coated quartz dust plus dead tubercle bacilli, and iron-coated quartz dust plus tuberculin.

Some of the essential findings are given in tabular form. An extensive pulmonary consolidation was produced by the iron-coated quartz dust in conjunction with dead tubercle bacilli. This consolidation consisted basically of a chronic accretive interstitial pneumonitis with extensive obliteration of air spaces, collagenous fibrosis, focal necrosis, calcification, and cavitation. The same dust in conjunction with tuberculin produced a similar consolidation although there was less collagenous fibrosis, less focal fibrosis, virtually no calcification, and no cavitation.

The pulmonary diseases produced by the combination of iron-coated dust with either living tubercle bacilli, dead tubercle bacilli, or merely tuberculin are very similar and differ from one another in minor detail only. These differences in relatively minor aspects are considered to be functions of host susceptibility or dosage of pathogenic agents, or both.

The ability of dead tubercle bacilli and of tuberculin, in conjunction with iron-coated quartz dust, to produce pulmonary disease simulating tuberculosilicosis is interpreted to support the thesis of Vigliani and Pernis that tuberculosilicosis is basically a reciprocal potentiation of the inflammatory response to quartz dust and to the tuberculous infection.

Sumario

Tuberculosilicosis Experimental: Comparación de los Efectos Producidos por Algunos de sus Varios Componentes Patógenos

Después de dividir en grupos a cobayos tuberculino-positivos se les inyectó intratraquealmente uno de los siguientes agentes patógenos o combinaciones de agentes: polvo de cuarzo recubierto de hierro, bacilos tuberculosos vivos, bacilos tuberculosos matados con formalina, tuberculina, polvo de cuarzo recubierto de hierro más bacilos tuberculosos vivos, polvo de cuarzo recubierto de hierro más bacilos tuberculosos muertos y polvo de cuarzo recubierto de hierro más tuberculina.

Algunos de los hallazgos esenciales se presentan en forma tabular. Con el polvo de cuarzo recubierto de hierro unido a bacilos tuberculosos muertos se produjo una extensa hepatización pulmonar. Esta hepatización consistió fundamentalmente en neumonitis intersticial acretoria crónica con extensa obliteración de los espacios aéreos, fibrosis colágena, esfacelamiento focal, calcificación y formación de cavernas. El mismo polvo combinado con tuberculina produjo una hepatización semejante aunque hubo menos fibrosis colágena, menos esfacelo local, apenas calcificación y fué nula la cavitación. Se describen las diferencias entre estos hallazgos y los notados en los grupos de testigos.

Las afecciones pulmonares producidas por la combinación de polvo recubierto de hierro ya con bacilos tuberculosos vivos, bacilos tuberculosos muertos o meramente tuberculina son muy semejantes y no discrepan entre sí más que en pequeños detalles. Estas diferencias en puntos relativamente secundarios se consideran como debidas a la susceptibilidad del huésped o la dosis de los agentes patógenos o a ambas causas.

Se considera que la capacidad de los bacilos tuberculosos muertos y de la tuberculina para producir, en combinación con el polvo de cuarzo recubierto de hierro, una neumopatía que simula tuberculosilicosis, presta apoyo a la tesid de Vigliani y Pernis de que la tuberculosilicosis es fundamentalmente una potenciación recíproca de la respuesta inflamatoria al polvo de cuarzo y a la infección tuberculosa.

Resume

Tuberculose expérimentale: Une comparaison des effets produits par quelques uns de ses différents composés pathogènes

On divisa en groupes des cobayes positifs à la tuberculine, et on leur injecta dans la trachée un des agents pathogenes suivants, ou une association d'agents: de la poussière de quartz recouvert de fer, des bacilles tuberculeux vivants, des bacilles tuberculeux tués au formol, de la tuberculine, des bacilles tuberculeux vivants associés à de la poussière de quartz recouvert de fer, des bacilles tuberculeux morts associés à de la poussière de quartz recouvert de fer, et de la poussière de quartz recouvert de fer associée à de la tuberculine.

Les auteurs donnent quelques unes des découvertes essentielles sous forme de classification. Une congestion pulmonaire étendue fut produite par la poussière de quartz recouvert de fer associée à des bacilles tuberculeux morts. Cette congestion consista surtout en une pneumonie interstitielle chronique infiltrante, avec une obstruction étendue des espaces aériens, en fibrose collagène, en nécrose focale, calcification et ulcération. La même poussière associée à de la tuberculine produisit une congestion semblable, bien qu'avec moins de fibrose collagène, moins de fibrose focale, pratiquement pas de calcification et pas d'ulcération. Ces différences entre les découvertes et les groupes de contrôle sont décrites par les auteurs.

Les maladies pulmonaires produites par la combinaison de poussières recouvertes de fer et de bacilles tuberculeux vivants ou morts, ou simplement de la tuberculine sont grandement semblables et ne diffèrent l'une de l'autre que par des détails mineurs. Les auteurs pensent que ces différences, sous des aspects relativement mineurs, sont fonction de la sensibilité de l'hôte ou de la dose d'agents pathogènes, ou fonction des deux à la fois.

Ils interprètent la possibilité des bacilles tuberculeux morts et de la tuberculine à produire, en association avec de la poussière de quartz recouverte de fer, une maladie pulmonaire simulant une tuberculosilicose, comme confirmation de la thèse de Vigliani et Pernis, qui disent que la tuberculosilicose est, à la base, une potentialisation réciproque de la réponse inflammatoire à la poussière de quartz et à l'infection tuberculeuse.

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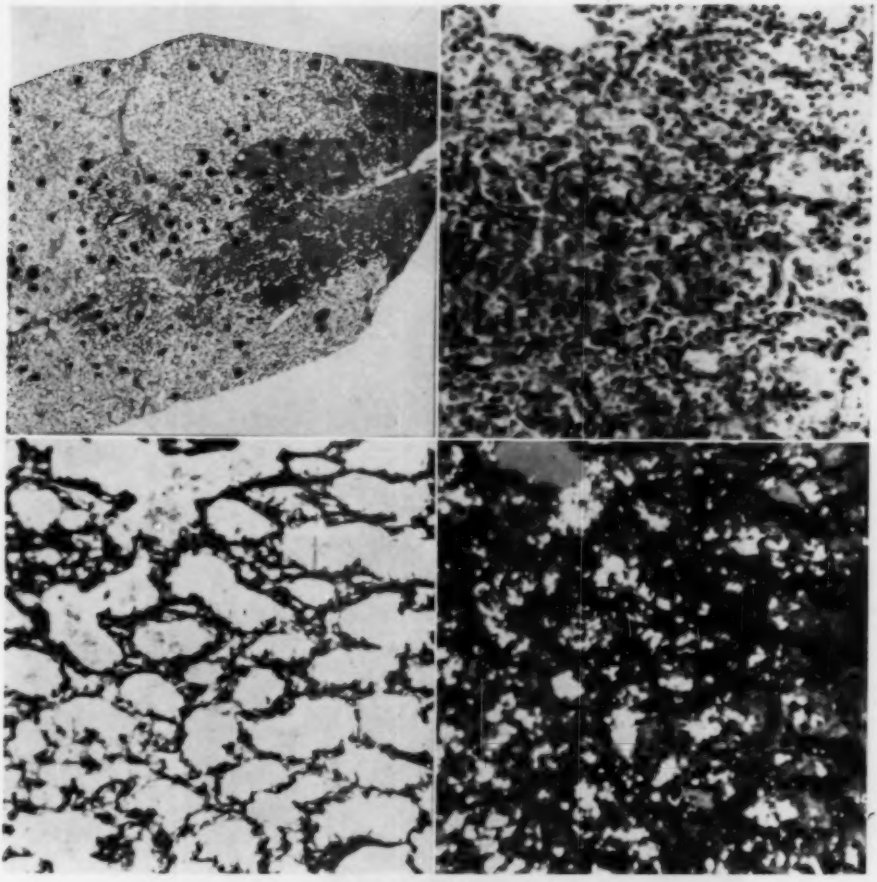
Figures 1–28 follow.



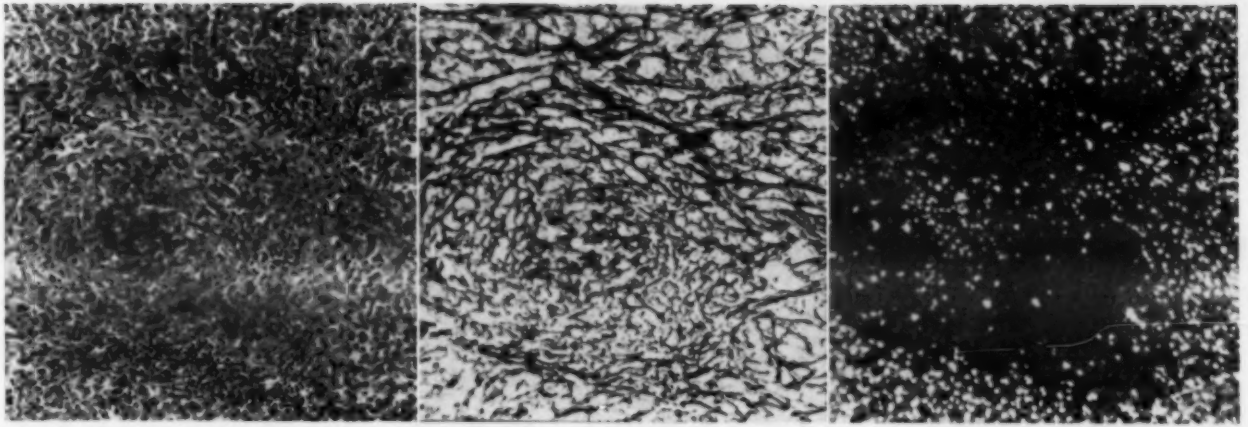
Dust Control

FIG. 1 (Upper left). Guinea pig No. 593, injected intratracheally with 150 mg. of iron-contaminated quartz dust, was killed nine and one-half months later. There is focal consolidation which is composed of irregular foci poorly separated by empty air spaces. The more densely staining small round foci are composed of lymphocyte-like cells and represent a nonspecific response of pulmonary tissue to a chronic irritant. (Hematoxylin and eosin, ×4.4)

FIG. 2 (Upper right). Guinea pig No. 594, injected intratracheally with 150 mg. of iron-contaminated quartz dust, died twelve months later. Although this field is very cellular, vague outlines of air spaces are still discernible. The cell outlines are generally poorly defined and the nuclei tend to be ovoid and vesicular. There is no collagen production and no tendency to produce nodules. (Hematoxylin and eosin, ×160)

FIG. 3 (Lower left). The same field as in figure 2 showing the reticulin pattern. There is very little obliteration of air spaces although there is universal thickening of septal walls. (Gordon and Sweets, ×160)

FIG. 4 (Lower right). Composite of the same field as in figure 2 showing the distribution of the silica. The mineral ash is situated mainly within air spaces in which it is intra- and extracellular. Some, but very little, silica is found in the septal walls. (×160)

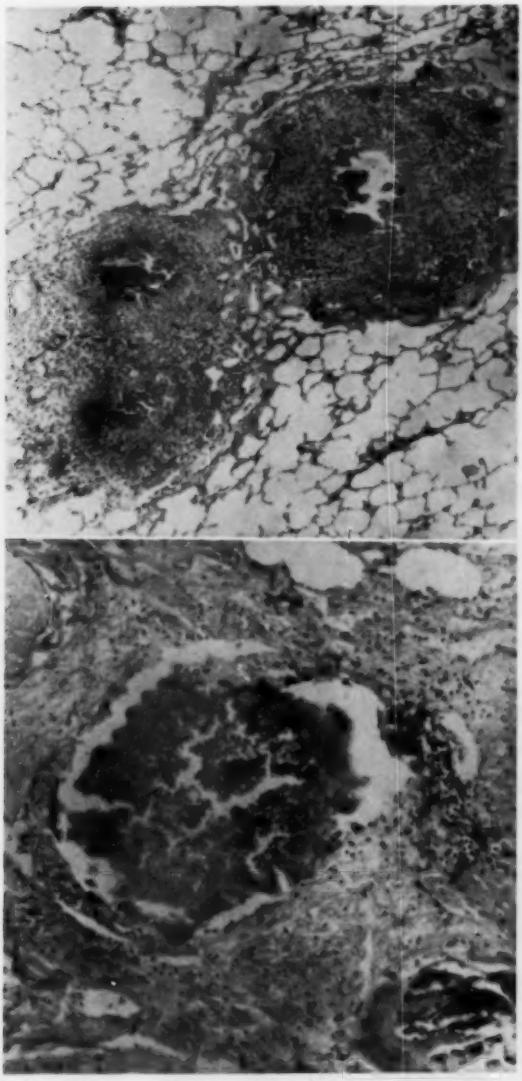


Dust Control

FIG. 5 (Left). Guinea pig No. 592, injected intratracheally with 150 mg. of iron-contaminated quartz dust, was killed nine and one-half months later. This field shows a nodule of crowded reticuloendothelial cells in one of the enlarged satellite lymph nodes. (Hematoxylin and eosin, ×89)

FIG. 6 (Center). The reticulin pattern of the same field as in figure 5 shows that the nodule is very poorly defined by an increased density of the fiber network. (Gordon and Sweets, ×89)

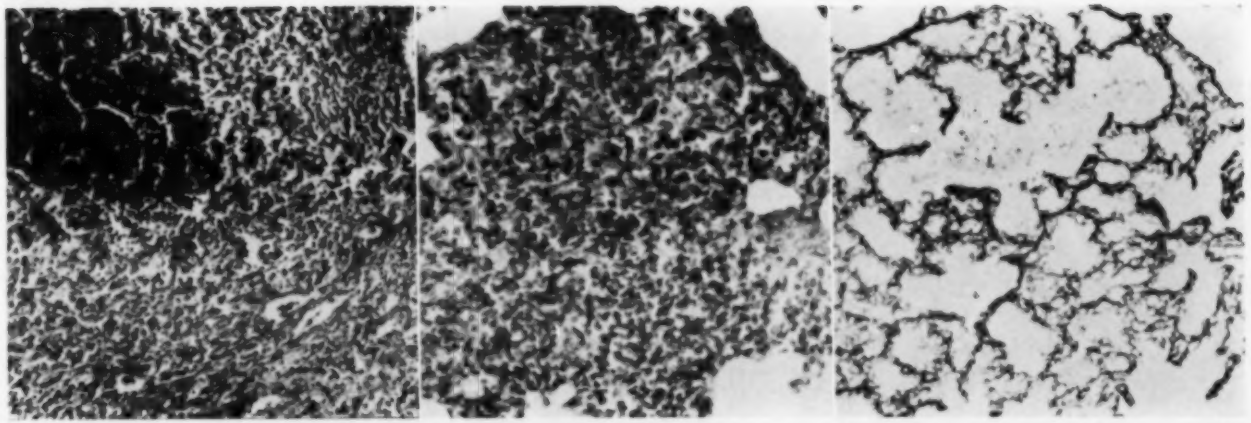
FIG. 7 (Right). The composite picture of the same field as in figure 5 shows a relatively small amount of dust in the center and practically no dust elsewhere in the nodule. However, abundant dust is present in the lymph tissue around the nodule. (×89)



Dead-Bacteria Control

FIG. 8 (Top). Guinea pig No. 517, injected intratracheally with 3 mg. of formalin-killed tubercle bacilli, was killed six months later. There are nodules of limited size, some of which tend to become confluent. The nodules consist of focal accretive chronic interstitial pneumonitis with necrosis and often too with calcification in the center of the foci. (Hematoxylin and eosin, ×46)

FIG. 9 (Bottom). Guinea pig No. 521, injected intratracheally with 3 mg. of formalin-killed tubercle bacilli, died twelve and one-half months later. This is the characteristic lesion of the group. The necrotic center is surrounded by a moderately narrow zone of chronic accretive interstitial pneumonitis with considerable collagen formation involving also nearby septal walls. The stigmata of a tuberculous inflammation are absent. (Hematoxylin and eosin, ×116)

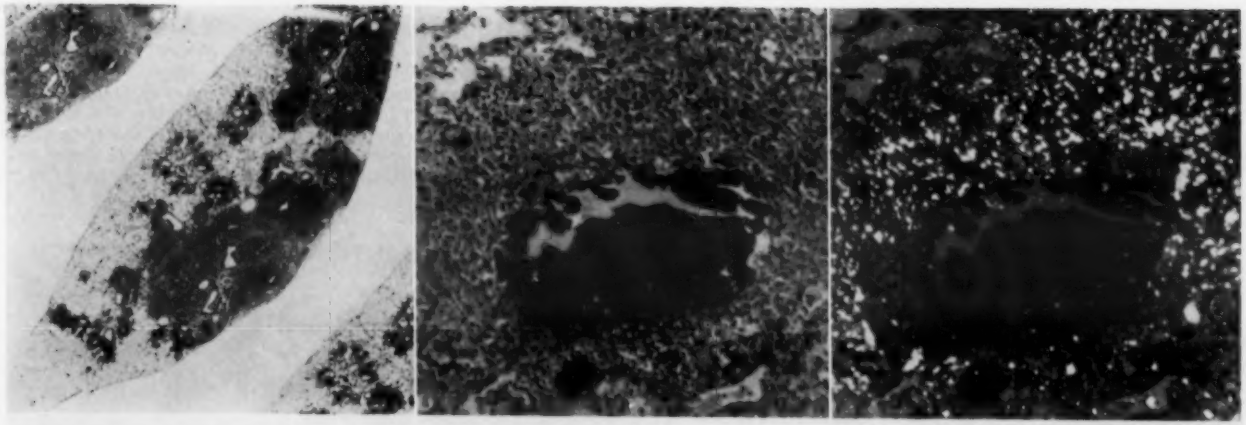


Living-Bacteria Control

FIG. 10 (Left). Guinea pig No. 676, injected intratracheally with 1 ml. of a 10^{-2} dilution of a broth culture of living tubercle bacilli, died four and one-half months later. This is the only animal in which necrotizing lesions were found. The necrosis occurred in the center of a focus of accretive chronic interstitial pneumonitis. The zones of epithelioid cells and of lymphocytic infiltration characteristic of a tuberculous granuloma are absent. No giant cells are present. (Hematoxylin and eosin, ×89)

FIG. 11 (Center). Guinea pig No. 699, injected intratracheally with 1 ml. of a 10^{-2} dilution of a broth culture of living tubercle bacilli, was killed fourteen months later. Foci of densely cellular accretive chronic interstitial pneumonitis were the more usual findings of this group. This lesion is well demonstrated in this field. (Hematoxylin and eosin, ×152)

FIG. 12 (Right). The reticulin pattern of the same field as is shown in figure 11 shows the obliteration of some of the air spaces and the thickening of septal walls. (Gordon and Sweets, ×152)

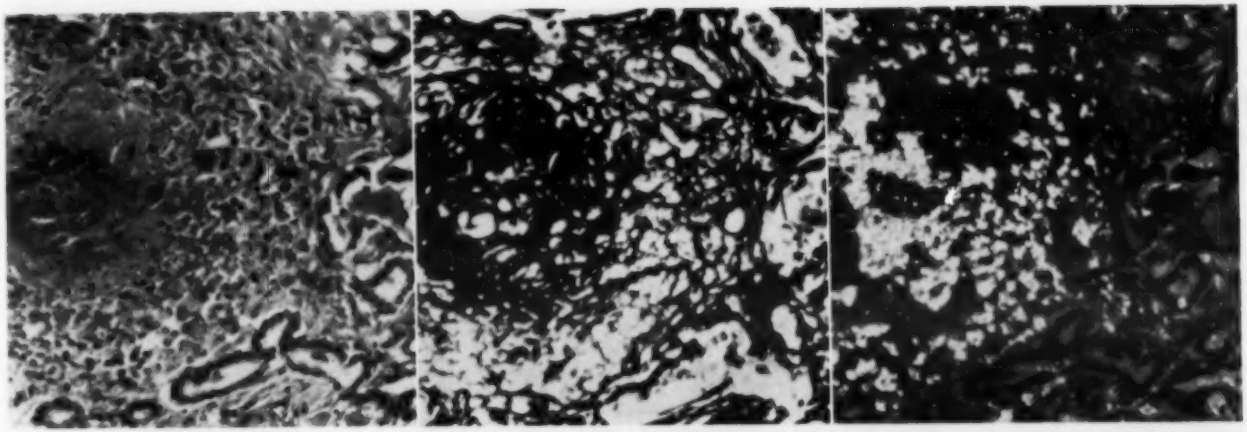


Dust-plus-Dead-Bacteria

FIG. 13 (Left). Guinea pig No. 480 was injected intratracheally with 3 mg. of dead bacteria and 75 mg. of iron-contaminated quartz dust. This animal died four months later. There is extensive and uniform consolidation with several foci of necrosis. In addition, there are several more isolated foci of consolidation which are riddled by uninvolved air spaces. These foci more closely resemble those of the pure dust lesion. (Hematoxylin and eosin, ×3.3)

FIG. 14 (Center). Guinea pig No. 490 was injected intratracheally with 3 mg. of dead bacteria and 75 mg. of iron-contaminated quartz dust. This animal died eight months later. There is a calcific, necrotic center in a moderately large region of accretive chronic interstitial pneumonitis. Very little collagen production is present. The stigmata of a tuberculous inflammation are absent. (Hematoxylin and eosin, ×89)

FIG. 15 (Right). Composite photograph of the same field as in figure 14 showing a moderate amount of well-distributed quartz dust in the tissues surrounding the necrotic zone. There is very little dust in the necrotic tissue. (×89)

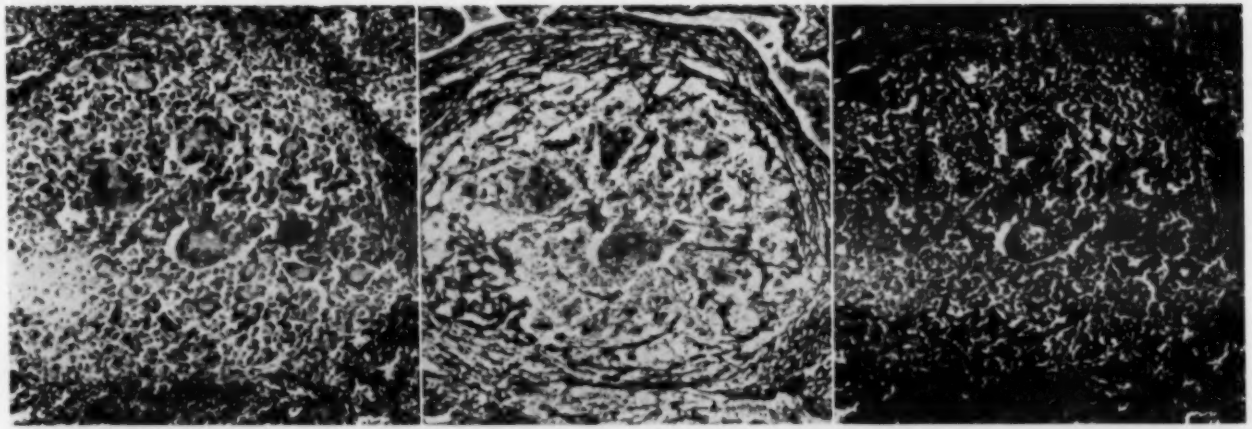


Dust-plus-Dead-Bacteria

FIG. 16 (Left). Guinea pig No. 636 was injected intratracheally with 1 mg. of dead bacteria and 50 mg. of iron-contaminated quartz dust. This animal died eleven months later. In this field there is central necrosis in a region of accretive chronic interstitial pneumonitis without calcification. On the periphery, there is advanced collagenization with metaplasia of the epithelium lining the residual air spaces, a process which has been loosely termed "adenomatosis." (Hematoxylin and eosin, ×152)

FIG. 17 (Center).The reticulin pattern of the same field as in figure 16 demonstrates the densification of the reticulin network which precedes the necrosis. Air spaces are obliterated except at the periphery. (Gordon and Sweets, ×152)

FIG. 18 (Right). Composite photograph of the same field as in figure 16 shows heavy concentrations of quartz dust in the necrotic zone and elsewhere except in the regions of "adenomatosis." The latter regions nearly always contain very little dust. Elsewhere, the distribution of dust is unpredictable. (×152)



Dust-plus-Dead-Bacteria

FIG. 19 (Left). Guinea pig No. 498 was injected intratracheally with 1 mg. of dead bacteria and 50 mg. of iron-contaminated quartz dust. The animal was killed thirteen months later. This field shows one of the nodules in a satellite lymph node composed of reticuloendothelial cells and Langhans' giant cells. This is similar to some of the nodules seen in lymph nodes of dust-control animals. (Hematoxylin and eosin, ×89)

FIG. 20 (Center). Reticulin pattern of the same field as in figure 19 showing paucity of reticulin within the nodule and crowding of circumferential fibers on the periphery. (Gordon and Sweets, ×89)

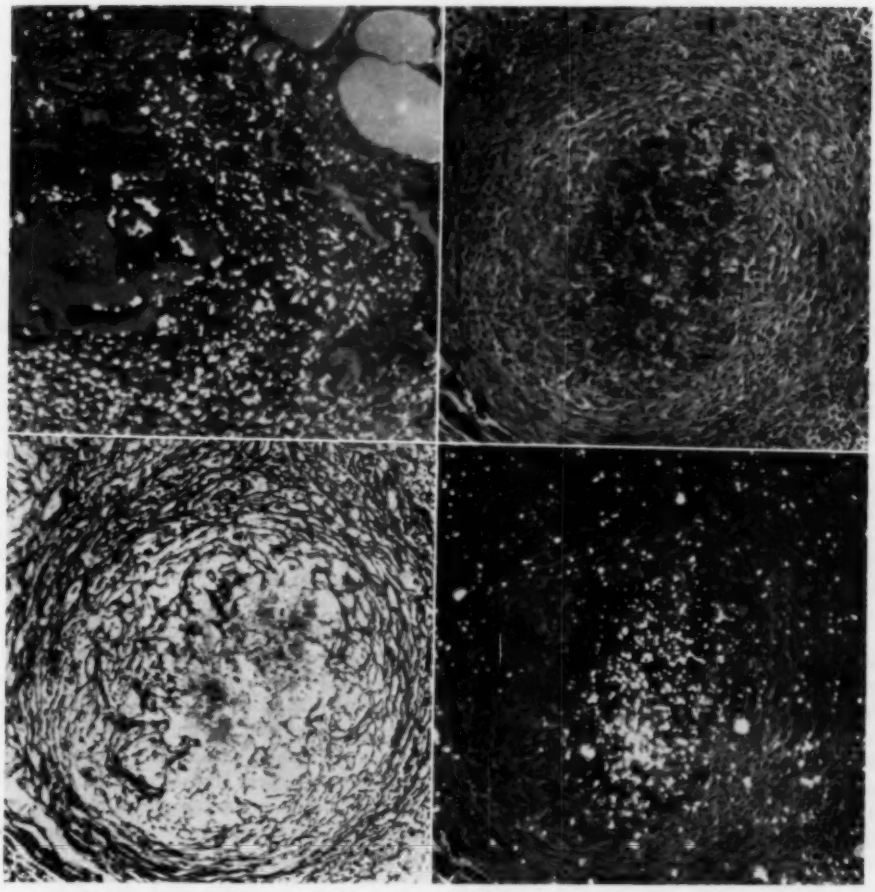
FIG. 21 (Right). Composite photograph of the same field as in figure 19 demonstrates that the giant cells and many of the reticuloendothelial cells contain quartz dust, indicating that their existence is a response to the presence of dust. (×89)

Dust-plus-Living-Bacteria

FIG. 22 (Left). Guinea pig No. 711 was injected intratracheally with 50 mg. of iron-contaminated quartz dust and 1 ml. of a 10^{-3} dilution of a broth culture of living tubercle bacilli. The animal was killed fourteen months later. There is extensive, uniform consolidation with interconnected, band-like areas of necrosis. The latter are marked by peripheral calcification. (Hematoxylin and eosin, ×4.2)

FIG. 23 (Center). Same animal as in figure 22. A higher magnification of the edge of a necrotic zone shows that the necrosis has occurred within a region of hyaline, dense collagen which still contains few scattered, pyknotic nuclei. The usual stigmata of a tuberculous inflammation are absent. (Hematoxylin and eosin, ×152)

FIG. 24 (Right). Guinea pig No. 727 was injected intratracheally with 50 mg. of iron-contaminated quartz dust and 1 ml. of a 10^{-3} dilution of a broth culture of living tubercle bacilli. The animal died four months later. This area of consolidation consists of accretive chronic interstitial pneumonitis with peripheral "adenomatosis" and central necrosis. The necrotic center is cavitating. (Hematoxylin and eosin, ×80)



Dust-plus-Living-Bacteria

FIG. 25 (Upper left). Composite photograph of the same field as in figure 24 shows well-distributed flocs of quartz dust throughout the consolidated zone, inclusive of the necrotic focus but exclusive of the region of "adenomatosis" where the dust is scanty. (×94)

FIG. 26 (Upper right). Guinea pig No. 707 was injected intratracheally with 50 mg. of iron-contaminated quartz dust and 1 ml. of a 10^{-2} dilution of a broth culture of living tubercle bacilli. The animal was killed eight months later. This field shows one of the many granulomatous nodules within enlarged satellite nodes. The nodule is composed of circumferentially arranged cells and fibers and a necrotic center. There are no giant cells or epithelioid cells. (Hematoxylin and eosin, ×94)

FIG. 27 (Lower left). The reticulin pattern of the same field as in figure 26 shows the characteristic three zones that were also seen in similar lymph nodal lesions in the *dust-plus-tuberculin* group of animals. There is an outer circumferential zone, a middle radial zone, and a central necrotic zone, largely devoid of fibers. (Gordon and Sweets, ×94)

FIG. 28 (Lower right). Composite photograph of the same field as in figure 26 showing abundant quartz dust in the center of the nodule and very little in the periphery. This is another example of the unpredictability of the dust distribution, since it is more common to find the reverse. (×94)

SKIN SENSITIVITY OF HUMAN BEINGS TO HISTOPLASMA CAPSULATUM AND BLASTOMYCES DERMATITIDIS POLYSACCHARIDE ANTIGENS[1, 2]

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INTRODUCTION

Skin-test antigens prepared from various systemic fungi have been in use for more than twenty years. In general, the most widely used of these antigens are the culture filtrates: histoplasmin prepared from *Histoplasma capsulatum*, coccidioidin from *Coccidioides immitis*, and blastomycin from *Blastomyces dermatitidis*. For all three, dosage is reckoned in terms of a dilution factor, such as a 1 to 100 or 1 to 1,000 dilution of the filtrate. Although the potency of filtrates may vary considerably from batch to batch, only in the case of histoplasmin has an officially recognized reference standard antigen been provided against which to match the potency, to "standardize," newly prepared products (1).

Experience with fungous antigens, as with mycobacterial antigens, leaves no doubt concerning the relation between dosage and the degree of the response. In subjects who are sensitive to a particular antigen, the larger of two doses produces the larger reaction. Quantitative measurement of dosage of an antigen in terms of weight of active substance would therefore seem an important goal, from both the scientific and the practical points of view. In the mycobacterial field, progress toward this goal was marked by the preparation of a purified protein derivative (PPD) of tuberculin (2) and, more recently, of other PPD antigens (3, 4). Even though PPD is not a chemically pure substance, the active principle of tuberculin has been identified as a protein or protein complex. The dose of PPD is therefore expressed as milligrams of protein per unit volume of diluent.

Corresponding progress in the fungus field is as yet inconspicuous, although not entirely absent. The most promising leads so far have been reported with polysaccharide extracts of fungous culture filtrates, particularly of *H. capsulatum* and *B. dermatitidis* (5–11). Trials of these antigens, however, have been limited almost entirely to experimentally infected laboratory animals and small numbers of human beings, usually patients with clinically active mycotic disease.

As reported earlier (12, 13), soluble polysaccharides extracted from yeast-phase broth cultures of *H. capsulatum* and *B. dermatitidis* and evaporated to dryness by desiccation compared favorably with histoplasmin and blastomycin in eliciting skin reactions in experimentally infected guinea pigs. A dose of 0.01 mg. of the dry powder appeared to give optimal results. Subsequent tests with the same dose in 134 human volunteers—hospital staff and medical students—gave results sufficiently encouraging to warrant a more extensive field trial of both antigens. The purpose of the present paper is to report the results of skin tests with the two polysaccharide antigens in 3,808 healthy young men.

Study Population and Procedures

For more than two years the Tuberculosis Program of the Public Health Service, in cooperation with the U. S. Navy, has been conducting a skin-testing program of recruits entering the naval training centers at San Diego and the Great Lakes. All recruits are tested routinely, within a few days after arrival at the centers, with tuberculin, histoplasmin, and other antigens. The two polysaccharide antigens, which are of interest for the present report, were given at different periods of time at the two centers during 1959 and in the spring of 1960. Altogether, 1,677 recruits (more at San Diego than at the Great Lakes) were tested with the *H. capsulatum* polysaccharide, and another group of 2,131 (about equal numbers at each center) were tested with the *B. dermatitidis* polysaccharide. Young men entering the Navy as recruits come from all parts of the country; most of them are between seventeen and twenty-one years of age; and 95 per cent are white.

[1] From the Tuberculosis Program, Public Health Service, U. S. Department of Health, Education, and Welfare, Washington 25, D. C.; the Department of Bacteriology, University of Utah; and the Veterans Administration Hospital, Salt Lake City, Utah.

[2] Aided by a grant from the Committee on Medical Research of the American Thoracic Society, medical section of the National Tuberculosis Association.

The skin tests are given by intracutaneous injection of 0.1 ml. of a dilution containing the desired dose of antigen. Most of the testing and all of the reading of reactions are done by Public Health Service field staff, each of whom has had intensive training and supervised field experience in skin-testing work. The sizes of the reactions are measured in millimeters of induration after forty-eight hours, as a rule; occasionally, after seventy-two hours. The measurements are recorded on individual record cards for each recruit.

The histoplasmin used in this program is a culture filtrate of *H. capsulatum*, identified as H-42, and given in a dilution of 1:100. Filtrate H-42 was prepared by Howell of the Public Health Service; its method of preparation, extensive field use, and adoption as a reference standard product by the Division of Biologic Standards, National Institutes of Health, have been summarized elsewhere (14). The *H. capsulatum* polysaccharide, prepared from strain G17M, and the *B. dermatitidis* polysaccharide, prepared from strain 6052, were both used in a dose of 0.01 mg. per 0.1 ml. Details of the preparation of both antigens, together with results of chemical tests, biologic assay in experimental animals, and initial trials in human beings, are given in earlier reports (12, 13).

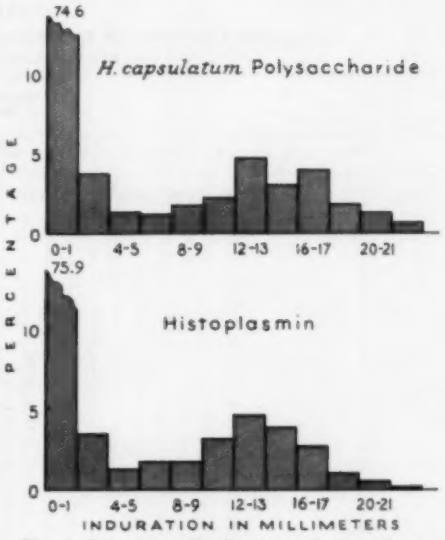

FIG. 1. Frequency distributions of sizes of reactions to *H. capsulatum* polysaccharide and histoplasmin in 1,677 naval recruits tested with both antigens.

RESULTS

Results with the 0.01-mg. dose of *H. capsulatum* polysaccharide and the 1:100 dilution of histoplasmin H-42 are shown in figure 1 as frequency distributions of sizes of reactions in 2-mm. groupings. The two histograms are very similar. Both are bimodal, roughly 80 per cent of the reactions comprising a sharply decending left-hand segment; the remaining 20 per cent, which range from about 6 to 26 mm. in size, are distributed in a form resembling the normal curve.

The principal difference between the two distributions is that reactions to the polysaccharide antigen are slightly larger than to the histoplasmin. The lowest frequency of reactions between the two segments of the distribution is 1.1 per cent at 6 to 7 mm. for the polysaccharide, and 1.3 per cent at 4 to 5 mm. for histoplasmin. The right-hand segment of the polysaccharide distribution, although its symmetry is somewhat marred by irregularities in the middle portion, appears to center around 14 to 15 mm.; the corresponding segment of the histoplasmin distribution centers at 12 to 13 mm. Actually, the mean size of reactions measuring 6 or more mm. is 14.1 mm. for the polysaccharide and 12.9 mm. for histoplasmin.

The correlation of sizes of reactions to the two antigens (table 1) indicates that most persons with little or no reaction to one antigen had little or no reaction to the other, and most of those who reacted to one antigen also reacted to the other. If the criterion for a positive reaction is set at 6 or more mm., for example, and at less than 6 mm. for a negative reaction, agreement between results of the two tests is 97.2 per cent: 311 of the 1,677 recruits (18.5 per cent) were positive to both antigens; 1,319 were negative to both. Among the remaining 47, or 2.8 per cent of the total group, 32 were positive to only the polysaccharide and 15 to only histoplasmin. It may be noted also that among the 1,272 recruits with no reaction (0 mm.) to histoplasmin, 25 had at least 6 mm. to the polysaccharide; while, among the 1,250 with no reaction to the polysaccharide, only 11 had at least 6 mm. to histoplasmin.

Reactions to the 0.01-mg. dose of *B. dermatitidis* polysaccharide antigen are plotted in figure 2. Most of the 2,131 recruits tested with this antigen had no reaction, some had a few millimeters of induration, and only 21 had reactions measuring from 6 to 18 millimeters. The result is a so-called inverted J-shaped distribution,

TABLE 1

CORRELATION OF SIZES OF REACTIONS TO HISTOPLASMIN AND H. CAPSULATUM POLYSACCHARIDE IN NAVAL RECRUITS

H. Capsulatum Polysaccharide G17M 0.01 Mg. Induration in Mm.	Histoplasmin H-42 1:100 Induration in Mm.																									Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26+	
0	1,184		18	23	8	6	1	3		1		3	2		1													1,250
1				1																								1
2	22		4	1	1	1																						29
3	26		5	2																								33
4	13		1				1	2																				17
5	2					1											1											4
6	1		1	1			2	2	1																			8
7	5			1			1						3	1														11
8	1							1		3		1	1	2					1									10
9	3				2	1	2	3		2	1	2	1	1	1													19
10	3							1		3	2	3	1		2			1		1								17
11	4									2	4	3	2		1	2	1	1										20
12	2							1		3	6	3	5	8	4	2	2	1	1									38
13	1							3	1	1	4	4	6	7	6	3	1	2	2									41
14	2						1	1		1		3	4		4	1	2	1			1	1						22
15								1	2	3	1	2	4	1	6	6	2											28
16	1				1		1	1	1		1	2	3	6	2	6	2	2	2	1	1	1						34
17	1									1		3	4	5	2	1	8	3	2		1		1					32
18												1	2	1	1	3	4	3	1		1							17
19									1	1		2	1	1		3	2	1			1							13
20													3	2		1	1	1	2	1								11
21								1		1					2		1	1		2			1					9
22												1			1	2				1	1							6
23	1														1			1			1							4
24																												—
25																												—
26+														2	1													3
Total	1,272	—	29	29	12	9	9	20	6	22	19	33	42	37	35	30	27	18	11	6	7	2	2	—	—	—	—	1,677

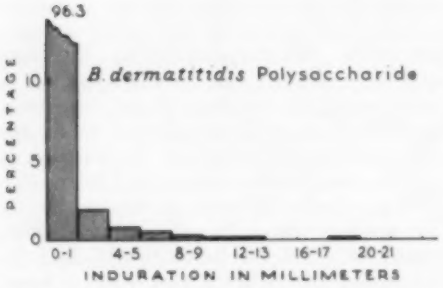

FIG. 2. Frequency distribution of sizes of reactions to *B. dermatitidis* polysaccharide antigen in 2,131 naval recruits.

with a very high frequency at 0 to 1 mm. (96.3 per cent), an abrupt drop to about 2 per cent at 2 to 3 mm., and progressively lower frequencies for increasing sizes of reactions. A distribution of this kind gives no clue as to what might be a reasonable criterion for classifying a reaction as positive, signifying a specific response caused by infection with *B. dermatitidis*, as differentiated from a negative reaction which signifies absence of such infection.

Despite the very low frequency of large reactions—only 1 per cent measured 6 or more mm.—the correlation of reactions to *B. dermatitidis* polysaccharide and to histoplasmin H-42 (table 2) provides some additional information. Of the 21 recruits with polysaccharide reactions of 6 or more mm., the reaction to histoplasmin was larger in 7, the same size in one, and smaller in the remaining 13. Residence histories for the 5 recruits with reactions of more than 10 mm. to the polysaccharide and 10 mm. or less to histoplasmin revealed that one had lived all his life in Illinois; one in New Hampshire; one in Missouri, then Oregon; one in California, then Oklahoma; and one in Mexico, then California.

TABLE 2

CORRELATION OF SIZES OF REACTIONS TO HISTOPLASMIN AND B. DERMATITIDIS POLYSACCHARIDE IN NAVAL RECRUITS

B. Dermatitidis Polysaccharide 6052 0.01 Mg. Induration in Mm.	Histoplasmin H-42 1:100 Induration in Mm.																									Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26+	
0	1,609	—	11	25	17	16	13	20	22	29	41	36	42	34	38	19	17	11	5	19	1	5	6	5	7	1	3	2,052
1																												—
2	5			3									1	1				1										11
3	24					1					2		1				1											29
4	5									1			1		1		2	2										12
5	3												1			1					1							6
6	3		1				1			1			1	1							1							9
7					1																							1
8																	1											1
9	2			1												1												4
10																											1	1
11	1																											1
12	1								1																			2
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19																												—
20																												—
Total	1,653	—	12	29	18	17	14	20	24	31	44	36	47	36	39	21	21	14	5	19	3	5	6	5	7	1	4	2,131

The distribution of reactions to histoplasmin in these 2,131 recruits is similar to the distribution for the other group shown in figure 1 and table 1.

DISCUSSION

Epidemiologic studies of the systemic mycoses are, in large measure, dependent on the availability of skin-test antigens that can be used to identify persons who have been infected by the corresponding organism. Coccidioidin played a major role in the recognition, during the early 1940's, of coccidioidomycosis as an endemic, sometimes highly prevalent infection in the southwestern part of the United States. Histoplasmin proved equally crucial in the discovery, a few years later, that histoplasmosis is a usually benign, highly prevalent infection in much of the east-central region of the country. Subsequent studies and skin-testing surveys have contributed to the present, fairly detailed knowledge of the geographic distribution and variations in prevalence of these two mycotic infections throughout the United States and many parts of the world.

Blastomycosis is a different matter. Clinical cases of disease, usually caused by *B. dermatitidis* in this country and *B. brasiliensis* in South America, can no longer be considered rare; yet almost nothing is known about the prevalence of blastomycotic infection and its geographic distribution. As a result, we are in the position of not knowing whether the clinical cases of blastomycosis, like those of coccidioidomycosis and histoplasmosis, represent only a small fraction of the total numbers of infected persons; or whether, unlike the other two, blastomycotic infection usually results in disease.

A major difficulty has been that skin-testing surveys for blastomycotic infection have not helped a great deal in clarifying the situation, primarily because of cross reactions between blastomycin and histoplasmin (15, 16). Palmer's study of 5,941 student nurses in 1947–1949 showed, for example, that all but 15 of 611 students who reacted to blastomycin also reacted to histoplasmin (unpublished). Results reported by investigators from Duke University with heat-killed Blastomyces vaccine were somewhat more satisfactory: among 1,380 persons included

in a community-wide survey in Pitt County, North Carolina, 38 persons reacted to the blastomycin skin test and only 11 of them also reacted to histoplasmin (17, 18). The Pitt County survey raised the question, though, of why no more than 2 per cent of the population (27 persons) had reactions suggestive of blastomycotic infection when, only a few months previously, blastomycosis had assumed epidemic proportions in the community: within a period of six weeks, mycologically proved disease had been diagnosed in 10 residents of Pitt County.

If infection with *Blastomyces dermatitidis* occurs in a benign, asymptomatic form in any sizeable number of persons in this country, and if such infection is associated with the development of skin sensitivity, it should be possible to study the epidemiology of blastomycosis through skin-test surveys. Because of doubts about the efficacy of both blastomycin and Blastomyces vaccine for this purpose, the possibilities of the polysaccharide type of antigen seemed worth investigating. Concomitant studies with the *H. capsulatum* polysaccharide were considered desirable, not only in order to obtain information about the new antigen. Since the two polysaccharide products had been prepared by the same technique and were found to possess similar chemical composition and similar biologic activity in experimentally infected animals (12, 13), it was thought that comparison of the reactivity of the *H. capsulatum* polysaccharide with standard histoplasmin in human beings would provide at least indirect evidence of the reactivity of the *B. dermatitidis* polysaccharide.

Present results indicate that the *H. capsulatum* polysaccharide is reactive in human beings and that it contains at least one component in common with histoplasmin, as the sensitivity detected by one antigen was detected equally well by the other in the present study group. In this respect, the results are in line with the conclusion of Cross and Howell (8), from their work in guinea pigs, that "... the active principle of histoplasmin is, at least in part, polysaccharide in nature." It is nevertheless possible that the small amount of protein in the polysaccharide antigen contributes to its reactivity, since the dose of 0.01-mg. dry substance used here is approximately 100 times more concentrated than the estimated amount of dry substance contained in 0.1 ml. of diluted histoplasmin. Moreover, despite the apparent similarity in results with the two antigens, they might behave quite differently in persons sensitized by other fungous infections which produce cross sensitivity to histoplasmin (19).

Results with the *B. dermatitidis* polysaccharide antigen, although essentially negative, indicate that cross sensitivity in histoplasmin reactors may not be the problem with the polysaccharide antigen that it seems to be with blastomycin. In other words, the polysaccharide antigen may be more specific than blastomycin. Moreover, if this antigen is as reactive in human beings as the *H. capsulatum* polysaccharide antigen—and there seems to be no evidence at the moment against thinking so—it would appear that benign blastomycotic infection must be far less prevalent in this country than histoplasmal infection. For, if benign blastomycotic infection occurs in human beings and produces skin sensitivity, the existence of an endemic focus, or even an area of moderately high prevalence, would presumably be reflected by a relative concentration of reactors from that region. The absence in the present study of such a concentration of reactors leads to the conclusion that high prevalence areas of blastomycotic infection, if there are any, are so localized geographically that their discovery will require skin-test surveys in much larger samples of the population from all parts of the country than were included in the present study.

Summary

Polysaccharide extracts of culture filtrates of *Histoplasma capsulatum* and *Blastomyces dermatitidis*, after preliminary trials in laboratory animals to determine optimal dosage, were used for testing a total of 3,808 naval recruits from all parts of the country. Simultaneous tests were also given with standard histoplasmin, H-42, in a 1 to 100 dilution.

The results with the *H. capsulatum* polysaccharide and the histoplasmin were so similar that the two antigens appeared to be quantitatively equal, or very nearly so, in the doses used in the present study population. Almost all of the persons who reacted to one antigen also reacted to the other antigen; and, conversely, most of the persons who did not react to one antigen did not react to the other. The conclusion is drawn that the polysaccharide antigen contains at least some of the same reactive substances as histoplasmin.

The frequency of reactions to the *B. derma-*

titidis polysaccharide was very low, about 1 per cent, compared with about 20 per cent to histoplasmin. As there is little reason to believe that the polysaccharide antigen was nonreactive, these results suggest that benign blastomycotic infection—if it exists at all—is far less frequent in this country than benign histoplasmal infection.

Sumario

Sensibilidad Cutánea de los Seres Humanos a los Antígenos de Polisacáridos del Histoplasma Capsulatum y del Blastomyces Dermatitidis

Después de ensayos preliminares para determinar la dosis óptima, usáronse extractos polisacarídicos de filtrados culturales del *Histoplasma capsulatum* y del *Blastomyces dermatitidis* para comprobar un total de 3,808 reclutas navales de todas partes de los Estados Unidos. Se ejecutaron además ensayos simultáneos con histoplasmina estándard, H-42, a una dilución de 1 por 100.

Tan similares fueron los resultados obtenidos con el polisacárido del *H. capasulatum* y la histoplasmina que los dos antígenos parecen ser iguales cuantitativamente o casi iguales, a las dosis usadas en la población estudiada. Casi todas las personas que reaccionaron a un antígeno también reaccionaron al otro; y, viceversa, la mayoría de los que no reaccionaron a uno tampoco reaccionó al otro. Se saca la conclusión de que el antígeno de polisacárido contiene a lo menos algunas de las mismas sustancias reactivas que contiene la histoplasmina.

La frecuencia de las reacciones al polisacárido del *B. dermatitidis* fué muy baja, aproximadamente 1 por ciento, comparada con aproximadamente 20 por ciento para la histoplasmina. Como hay pocos motivos para creer que el antígeno del polisacárido no fuese reactivo, sugieren estos resultados que la infección blastomicótica benigna -si es que existe—es mucho menos frecuente en los Estados Unidos que la infección histoplásmica benigna.

Resume

Sensibilité cutanée des êtres humains aux antigènes polysaccharides d'Histoplasma capsulatum et de Blastomyces dermatitides

Les auteurs utilisèrent, pour expérimenter sur un total de 3.808 recrues de la marine, provenant de toutes les parties du pays, des extraits de polysaccharides de filtrats de cultures *d'Histoplasmasma capsulatum* et de *Blastomyces dermatitidis*, après des essais préliminaires sur des animaux de laboratoire pour déterminer le dosage optima. Ils expérimentèrent aussi en même temps avec de l'histoplasmine standard, H-42, à une dilution de 1%.

Les résultats obtenus avec le polysaccharide de *H. capsulatum* et l'histoplasmine furent tellement semblables que les deux antigènes semblent être quantitativement égaux, ou presque, aux doses utilisées dans la présente étude de la population. Presque toutes les personnes qui réagirent à un antigène réagirent aussi à l'autre; et, inversement, la plupart des personnes qui ne réagirent pas à l'un, ne réagirent pas à l'autre antigène. On en a tiré la conclusion que l'antigène polysaccharide contient au moins quelques unes des mêmes substances que l'histoplasmine.

La fréquence des réactions au polysaccharide de *B. dermatitidis* fut trés basse, à peu près 1%, comparée aux 20% approximatifs de l'histoplasmine. Comme il y a peu de raison de croire que l'antigène polysaccharide ne réagit pas, ces résultats font penser qu'une infection bénigne blastomycotique—autant qu'elle existe—est beaucoup moins fréquente dans ce pays que l'infection bénigne à histoplasmose.

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PASSIVE TRANSFER OF ACQUIRED RESISTANCE TO INFECTION WITH MYCOBACTERIUM TUBERCULOSIS BY MEANS OF CELLS[1, 2, 3]

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Introduction

Passive transfer of acquired immunity or hypersensitivity from responsive donors to normal recipients is considered a crucial test for the elucidation of the mechanisms by which immunity or hypersensitivity is established. Thus, on the basis of such experiments it is possible to discriminate the Arthus from the delayed type of hypersensitivity, the former being transferred by serum containing precipitating antibody and the latter by lymphocytes or mononuclear phagocytes derived from sensitive donors (1). Likewise, the importance of circulating antibody in acquired immunity to pneumococcal infection was demonstrated by the transfer of specific antibody-containing serum into man or experimental animals. Using similar techniques for the exploration of acquired immunity in tuberculosis, results have been obtained which indicate that no single protective factor alone seems responsible for postinfection or postvaccination immunity. Transfer of serum from infected or vaccinated donors to normal recipients resulted in increased resistance to challenge infection in some cases (2) and had no effect in others (3).

It was also found that virulent tubercle bacilli ingested by mononuclear cells from vaccinated donors had a reduced capacity to establish an infection in the anterior chamber of the eye of normal recipients when compared with bacilli ingested by mononuclears from normal animals (4). Further experiments with cultured mononuclears suggested that circulating and cellular factors played a combined role in the immune response to tuberculous infection (5, 6). To test the significance of these findings, experiments were undertaken in which cells from immunized donors were transferred to normal recipient animals. Various criteria were applied to estimate the effect of the transfers on subsequent challenge infection of the recipients.

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[2] This work was supported by research grant E-1302 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[3] Presented in part at a meeting of the New York Academy of Sciences, April 28–30, 1960 (16).

Materials and Methods

Animals: Male and female Swiss white mice of the Schwentker and the Webster strains, weighing between 17 and 20 gm., were used. Guinea pigs of a mixed breed weighed from 350 to 500 gm. They were fed with commercially available pellets. The guinea pigs were fed greens twice weekly.

Tubercle bacilli: Two attenuated strains were available, BCG (from the Henry Phipps Institute in Philadelphia, Pennsylvania) and R1Rv. The virulent strains were H37Rv and Vallée. (The three latter strains were originally from Dr. R. J. Dubos's laboratory, The Rockefeller Institute, New York, New York.) All strains were transferred weekly in Dubos Tween®-albumin liquid medium and were between seven and ten days old when used for the experiments. Stock cultures were also maintained on Löwenstein-Jensen medium and were transferred bimonthly.

Vaccination: Guinea pigs were vaccinated by the intracutaneous route with either BCG or R1Rv. In some cases BCG was reinjected approximately four weeks after the first injection. Mice were vaccinated by intravenous injection of 0.2 ml. of BCG culture. The donors were used between three to five weeks after vaccination.

Transfers: Cells from peritoneal exudates and lymph nodes were used in experiments with guinea pigs, and exudate and spleen cells with mice. Peritoneal exudates were elicited by intraperitoneal injection of 12 per cent sodium caseinate suspension. The cells were harvested four or five days later. Lymph nodes and spleens were teased with needles, and the freed cells were suspended in Hanks' balanced salt solution. Large clumps were allowed to settle and the cells remaining in the supernatant fluid were washed once by slow-speed centrifugation and resuspended in Hanks' balanced salt solution for immediate injection into the recipients. Injections of cells were done intraperitoneally or intracardially into guinea pigs and intraperitoneally into mice. The schedules of injections are given in the experimental section of this report.

Evaluation of effect of cell transfers: The following criteria were applied to measure the conse-

quence of transfers on the recipients' resistance to challenge infection: (*1*) degree of intracellular multiplication of R1Rv within cultured mononuclear cells derived from recipient guinea pigs, (*2*) evolution and involution of skin lesions in guinea pigs in response to intradermal injections of graded amounts of BCG, (*3*) extent of lesions induced in mice by the intravenous injection of R1Rv or small numbers of H37Rv and quantification of the bacillary population in the lungs, and (*4*) the response of mice to infection with a virulent strain of tubercle bacilli.

Viable tubercle bacilli in cell suspensions: Samples of cell suspensions used for transfers were inoculated on oleic-acid-albumin agar plates. Viable tubercle bacilli were recovered regularly from suspensions of splenic cells, but irregularly from suspensions of monocytes.

Results

Multiplication in vitro *of R1Rv within guinea pig mononuclear phagocytes derived from recipients of cells from vaccinated and normal donors:* In the first experiments, exploring the possibility of transferring a protective factor from vaccinated donors, an *in vitro* system was used for evaluation. At first, donor cells were injected into the peritoneal cavity which was then washed out four days later to obtain mononuclears for tissue culture. Subsequently, intracardial injections of donor cells were done, and a peritoneal exudate was elicited in the recipient animals by injection of glycogen solution. Simultaneously with the collection of lymph node cells for transfers, exudate mononuclears were also obtained from some of these donors for culture studies. The technique for setting up the infected mononuclear cultures and the mode of evaluation of intracellular multiplication of tubercle bacilli were described in detail in an earlier paper (7).

The procedures for these experiments were as follows:

Two vaccinated and two normal donor animals were injected intraperitoneally with a solution of glycogen. Four days later the animals were bled to death and the mononuclears in the exudate were collected for cultivation. Axillary, inguinal, mesenteric, and cervical lymph nodes were removed from these animals and from one additional normal and vaccinated animal. The cells were obtained by teasing the nodes and were washed once. Comparable amounts of cells (20 to 50 million) from normal and immunized donors were each injected intraperitoneally into a normal recipient. Cells were collected from the peritoneal cavities of these recipient animals four days later and were cultured with R1Rv *in vitro*. Slide cultures were removed at intervals, and intracellular multiplication was evaluated by counting 100 infected cells and grouping them according to the number of stainable bacilli which they contained. In other experiments the collected lymph node cells from the donors were injected intracardially. A glycogen exudate was then produced in these recipients, and the cells were collected and cultured *in vitro* four or five days later. Results of one such experiment are given in table 1.

As may be seen, exudate mononuclears from animals which had received lymph node cells from previously vaccinated donors exerted some inhibition on intracellular tubercle bacilli *in vitro*. This inhibition was independent of whether the donor cells were injected into the heart or the peritoneal cavity. No attempt was made to determine whether the cells from the peritoneal cavity of the recipient were the recipients' or the donors' cells or a mixture of both. These experiments were repeated with mice by transferring splenic cells instead of lymph node cells; the results were similar to those obtained with guinea pigs. However, multiplication of R1Rv within mouse mononuclear cells appeared to be slower than in those from guinea pigs. Therefore, the difference observed was smaller in the experiments with mononuclears from mice.

Development of dermal lesions in response to BCG in guinea pigs that received cells from normal and vaccinated donors: After the *in vitro* experiments had shown that some protective factor could be transferred from vaccinated donor to normal recipient animals by means of cells, it was important to determine whether the tissue response to an infection would be altered by repeated injections of cells from vaccinated donors. The lesions that developed after intradermal injection of attenuated strains can easily be followed over a prolonged period of time. According to previous experience, this technique allows the measurement of small differences of host resistance (8) and of residual virulence of tubercle bacilli of various degrees of attenuation (9).

Ten guinea pigs were vaccinated with BCG by the intradermal route. Two months later transfers were begun. Lymph node and splenic cells from two vaccinated donors were collected and pooled for each transfer. Cells from normal animals were similarly obtained. Cells were washed and suspensions were adjusted to contain from 1.5 to 3.0 $\times 10^8$ cells per ml.; 2 ml. of these two lots of cells were each injected intraperitoneally into 2 guinea pigs. Two additional animals received saline by the

TABLE 1

INTRACELLULAR MULTIPLICATION OF R1Rv WITHIN GUINEA PIG MONONUCLEAR PHAGOCYTES CULTURED IN VITRO

(The Mononuclear Cells Were Derived from Normal and Vaccinated Donors and the Recipients Were Injected Intraperitoneally (*A*) or Intracardially (*B*) with 25 × 10^6 Lymph Node Cells of the Former)

Preparation of Donor	Days of Cultivation	Donor Cells					Recipient Cells									
							A					B				
		Per Cent of Cells with Bacilli*				Average†	Per Cent of Cells with Bacilli				Average	Per Cent of Cells with Bacilli				Average
		1–2	3–5	6–10	>10		1–2	3–5	6–10	>10		1–2	3–5	6–10	>10	
None	1	62	31	7	0	2.4	54	33	11	2	3.0	65	29	6	0	2.3
	5	13	29	42	16	7.1	13	29	46	12	6.8	35	52	11	2	3.6
	8	5	12	27	56	11.1	9	14	27	50	8.3	7	13	27	53	10.7
BCG	1	62	33	5	0	2.3	58	33	9	0	2.6	63	30	6	1	2.5
	5	57	32	10	1	2.8	50	31	15	4	3.5	53	26	18	3	3.5
	8	67	21	6	6	2.9	47	29	16	8	4.1	55	21	13	11	4.1

* Per cent of mononuclear cells containing the indicated number of stainable bacilli. The figures are averages of counts done on duplicate or triplicate cultures.

† Average number of bacilli per infected phagocyte.

TABLE 2

THE DEVELOPMENT OF SKIN LESIONS* IN RESPONSE TO INTRADERMAL INJECTIONS OF BCG IN NORMAL GUINEA PIGS AND IN GUINEA PIGS RECEIVING CELLS FROM NORMAL AND VACCINATED DONORS

Group	Dilution of BCG	Days after Infection					
		1	2	8	29	38	61
A, Control (saline)	Undiluted	2.0/1.0	2.0/2.0	3.0/3.0	4.0/3.5	4.5/4.0	3.0/4.0
	10^{-1}	1.0/0.5	1.0/1.0	2.0/1.0	2.0/3.0	2.0/2.0	1.0/4.0
	10^{-2}	1.0/0.5	1.0/1.0	0.5/0.5	1.5/1.0	0.5/1.0	tr./1.5
	10^{-3}	0.5/0.5	1.0/1.0	0.5/0.5	1.0/1.0	0/tr.	tr./tr.
B, Cells from normal donors	Undiluted	1.0/1.5	2.0/2.0	3.0/2.0	3.5/4.0	3.0/4.0	4.0/3.5
	10^{-1}	0.5/1.0	1.0/1.0	1.5/2.0	3.0/3.5	2.0/3.0	3.0/2.0
	10^{-2}	0.5/0.5	1.0/1.0	1.0/1.0	2.0/2.0	1.0/1.5	0.5/2.0
	10^{-3}	0/0.5	0.5/1.0	0/1.0	0/1.0	tr./tr.	tr./0
C, Cells from vaccinated donors	Undiluted	1.5/1.0	1.0/1.0	0.5/1.5	2.0/2.0	2.0/2.0	1.0/2.0
	10^{-1}	1.0/0.5	1.0/1.0	0.5/1.0	1.5/1.0	1.0/0.5	2.0/1.0
	10^{-2}	1.0/0.5	0.5/0.5	0/0	0/0	tr./0	tr./tr.
	10^{-3}	0.5/0.5	0.5/0.5	0/0	0/0	0/0	0/0

* The readings were done in a "blindfolded" manner and were recorded in arbitrary units from 1 to 4. (tr = trace; 1 and 2 = nodular lesion without visible necrosis; 3 = nodular lesion with necrosis; 4 = ulcer)

same route. Cell transfers were repeated on the fourth, ninth, twelfth, and sixteenth days of the experiment. Care was taken to inject equal amounts of cells from normal and vaccinated donors. All recipient animals were albino female guinea pigs. On the fifth day of the experiment all 6 animals were injected intradermally on the back with 0.1 ml. of four dilutions of a Tween-albumin culture of BCG, namely, undiluted, 10^{-1}, 10^{-2}, and 10^{-3}. The skin reactions were recorded in arbitrary units.

The results of this experiment are represented in table 2. It is apparent that the lesions were

smaller in the animals that received cells from vaccinated donors than in those receiving either saline or cells from normal donors. There was no indication of a Koch phenomenon in the animals being injected with cells from vaccinated animals.

The evolution of a chronic infection in mice that received cells from normal and vaccinated donors: The results of the experiments to be described essentially confirm the above findings, but they also indicate the limitation of the degree of protection obtained by means of cellular transfers.

In the first experiment, donor cells were collected by teasing spleens of 10 normal and 10 BCG-vaccinated mice for each transfer. From 2.0 to 3.0 $\times$ 10^8 cells were injected intraperitoneally five times at intervals of four days. Control groups consisted of normal animals which received saline injections and others which had been vaccinated with BCG. Each group contained 5 animals. On the day of the second transfer, all mice were infected intravenously with 0.1 ml. of a culture of R1Rv. Four weeks later the animals were killed with chloroform. The spleens were removed and weighed; the lungs were removed and fixed with Zenker's solution. Sections of the lungs were stained with carbolfuchsin containing Tween 80 and counterstained with hematoxylin. The sections were scored "blindfoldedly" for extent and severity of lesions. The bacillary population in the lung lesions was determined by counting 100 mononuclear cells containing bacilli and grouping them according to the number of bacilli they contained. This technique was reported as valuable in estimating the intracellular parasitization of tissues, including information on the extent of the lesions caused by the organisms (10, 11).

TABLE 3

EXTENT OF PULMONARY TUBERCULOSIS AND OF BACILLARY POPULATION IN PULMONARY LESIONS AFTER INFECTION WITH R1Rv IN MICE WHICH RECEIVED CELLS FROM NORMAL AND VACCINATED DONORS*

Group	Lung Scoring†	Per Cent of Cells with Bacilli				Average‡
		1–2	3–5	6–10	>10	
Saline	100	9	20	31	40	7.3
Recipients of cells from normal donors	103	9	19	30	42	7.5
Recipients of cells from vaccinated donors	56	33	31	19	17	4.8
BCG-vaccinated	12	44	31	17	8	3.8

* Average figures from 5 animals in each group

† Microscopic evaluation of sections of lungs, setting the value of controls as 100. The following criteria were used: consolidation of lung parenchyma, perifocal reaction, and edema.

‡ *See* legend of table 1.

A summary of the results is given in table 3. The extent of infection and the severity of tissue reaction in recipients of cells from vaccinated donors were reduced when compared with those in the control group, but they were greater when compared with those in the BCG-vaccinated group. These findings were confirmed in a second experiment, using a small inoculum of H37Rv for the challenge infection.

In a second experiment, at the time of immunization, the donors were eighty-nine days old and the mice of the experimental groups were thirty-five days old. All donors were injected intravenously with 0.1 ml. of a culture of BCG; at the same time 10 mice of the experimental group were also vaccinated (*group B*). Monocytic peritoneal exudate cells were obtained from donors between thirty-four and forty-six days after vaccination. One group of recipients was injected with cells on days 1, 4, 8, and 13 of the experiment (*group C*) and a second group on days 4 and 8 (*group D*). The number of cells injected with each transfer varied from 1.2 to 1.5 $\times$ 10^7 per mouse, amounting to a total of 5.6 $\times$ 10^7 cells per mouse in *group C* and 2.9 $\times$ 10^7 in *group D*. All experimental animals were infected on the sixth day of the experiment, that is, thirty-eight days after BCG vaccination of *group B*, two days after the second transfer in *group C* and the first transfer in *group D*, respectively. The animals were killed thirty-eight days later. The evaluation of the infection was done as in the preceding experiment. When counting 100 infected mononuclear cells in the lesions, the number of oil-immersion fields which had to be searched was also recorded. It was found that this criterion gave a rather accurate picture of the spread of the infection. The histopathologic evaluation of the coded section was kindly done by Dr. Guido Majno, Department of Pathology, Harvard Medical School. The parameters used were consolidation of the lung parenchyma, perifocal reaction, and edema. Each of these was rated from 1 to 10, giving a maximal total of 30 for each section.

A summary of the results is given in table 4. The table contains the arithmetic means of values in every group and the standard deviation. The p values were obtained by doing "Students" t test for significance for all experimental groups. These results are also graphically represented in figure 1.

The data presented indicate that the bacterial

TABLE 4

THE EFFECT IN MICE OF VACCINATION WITH BCG AND THE INJECTION OF CELLS FROM VACCINATED DONORS UPON THE ESTABLISHMENT OF A CHRONIC INFECTION WITH H37Rv

Group	Lung Scoring*	Number and Distribution of Bacilli					
		Per Cent of Mononuclear Phagocytes Containing Bacilli†				Average†	Number of Fields‡
		1-2	3-5	6-10	10		
A, Controls: saline	100	34.6	39.44	18.83	7.17	4.2	103
		±3.26	±1.56	±3.26	±1.56		±14
B, BCG-vaccinated	73	70.75	26.1	2.75	0.4	2.0	2,546
		±2.34	±2.04	±0.66	±0.74		±826
P		<0.001	<0.001	<0.001	<0.001		<0.01
							>0.001
C, Transfer: 4 injections	79	56.2	30.3	11.0	2.6	2.9	383
		±3.78	±2.24	±1.48	±1.04		±119
P		<0.001	<0.001	>0.05	<0.001		<0.001
				<0.1			
D, Transfer: 2 injections	—	44.40	37.4	14.8	3.5	3.5	235.9
		±3.37	±1.22	±2.25	±1.17		±72.59
P		>0.05	>0.2	>0.2	>0.1		>0.1
		<0.1	<0.4	<0.4	<0.2		<0.2

* The values obtained in the scoring are converted to a scale setting the control value at 100. For details *see* text.

† *See* table 1.

‡ This figure represents the number of oil-immersion fields which had to be searched to find 100 mononuclear phagocytes containing stainable tubercle bacilli.

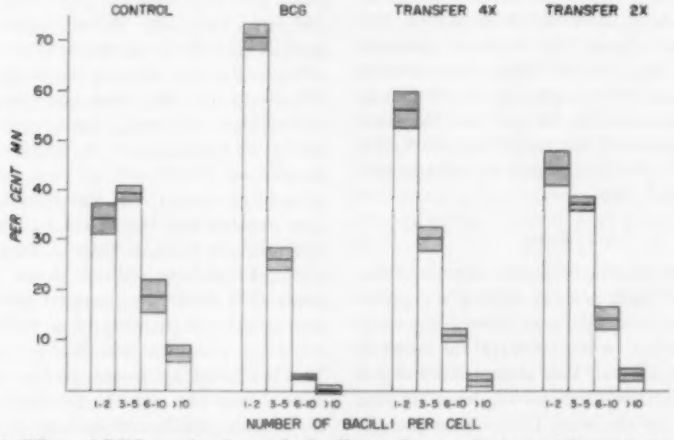

Fig. 1. Effect of BCG vaccination and of cell transfers on the bacterial population in the lungs of mice established five and one-half weeks after infection with H37Rv. The stippled areas indicate ± one standard deviation from the mean. For details *see* legend of table 4 and text.

population in lung tissues of mice vaccinated with BCG or receiving four injections of cells from immunized donors was reduced compared with that in normal controls. Likewise, the extent and severity of the pulmonary lesions showed a similar pattern. The lesions in animals of the experimental groups exhibited less consolidation and edema. The extent of perifocal reaction, how-

ever, was similar in all groups. Resistance to infection of animals that received cells from vaccinated donors four times was not as great as in the BCG-vaccinated group. Two injections of cells had less effect than four injections, and the difference between this and the control group was below the level of significance.

The progress of a virulent infection in mice that received cells from vaccinated donors: In a further experiment a test was made to determine whether the progress of a virulent infection would be influenced by cells from vaccinated donors in a manner similar to that of an attenuated infection.

The recipient animals were injected intraperitoneally with a total of approximately 300×10^6 cells derived from peritoneal exudates of donors which had been vaccinated with BCG twenty to thirty days earlier. Two days after the third transfer, the recipient animals, normal controls, and the animals vaccinated with BCG were injected intravenously with 0.1 ml. of a culture of the Vallée strain.

The mice of the recipient group died at the same rate as the normal controls, whereas the BCG-vaccinated animals survived for a considerably longer period of time. This experiment was repeated using an inbred strain of mice, C57 black, obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine. Again, transfer of cells from BCG-vaccinated donors had no effect upon the survival time of the recipients when challenged with the virulent strain Vallée. Direct BCG vaccination proved effective in prolonging survival time.

Discussion

There is convincing evidence that infection with tubercle bacilli or with some other pathogenic microorganisms induces altered reactivity of the phagocytic system toward the same or other microorganisms. This altered reactivity is manifested in many ways and can have different consequences for the host. Thus, components of Old Tuberculin exert a specific *cytotoxic effect in vitro* upon phagocytes from tuberculous animals. However, it has recently been reported that these cells are similarly more sensitive to inhibitory effects exerted by endotoxin from gram-negative organisms (12). This would indicate that phagocytic cells, and possibly other cells, undergo changes during the tuberculous infection which render them more sensitive or reactive to a variety of stimuli. Furthermore, mononuclear phagocytes from tuberculous animals exhibit altered behavior when infected *in vitro* with tubercle bacilli. This can be manifested either by a capacity to restrict intracellular multiplication of tubercle bacilli (6, 13, 14) or by greater resistance of these cells to the destructive effect exerted by intracellularly multiplying virulent tubercle bacilli (5).

The participation of a humoral factor in this increase of resistance has been described. This factor is not a gamma-globulin and presumably not an antibody. It also lacks specificity (5). However, these changes could not be observed in all instances (15). At present no satisfactory explanation is available for this discrepancy of findings except that the suppression of intracellular infection is manifested only when a small number of the cells is infected with a single bacillus, whereas it is overcome in cultures in which a majority of cells is infected with numerous bacilli (16). Again there is evidence of cross-immunity when cells derived from animals infected with brucellae are tested *in vitro* with bacilli (5).

The transfer experiments reported in this paper give additional evidence that some factor associated with cells of immunized or infected animals contributes to the state of increased resistance observed after primary infection with tubercle bacilli. These experiments also confirm and enlarge the findings made by Lurie (4). In the latter experiments, the virulent organisms were exposed *in vitro* to the monocytes from the immunized animal, and the infected cells were then injected into the anterior chamber of normal recipient animals, thus causing a relatively localized infection. More recent findings by Sever (17) showed a beneficial effect of the injection of exudate cells from BCG-immunized donors on a virulent infection in recipient mice. This is in contrast to the present results which showed that the protection transferred by cells from immunized donors was limited to attenuated or chronic infection and was not observed if the recipients were infected with a dose of virulent bacilli causing a rapidly progressing and fatal infection.

Some components associated with serum or plasma seem to contribute to acquired resistance in tuberculosis, as shown in *in vitro* and *in vivo* experiments. Thus, an inhibitory effect upon tubercle bacilli *in vitro* by serum from infected

animals was noted by several investigators (18, 19). The serum component behaved electrophoretically like an alpha-globulin and its activity was dependent on the presence of calcium ions. Furthermore, the appearance of this inhibitory property after infection was not suppressed by X-irradiation which abolished the antibody response (19). Transfer of immunity with serum has been successful in only a few experiments (2, 3). Undoubtedly, acquired immunity to tuberculosis is a complex phenomenon and is possibly mediated by humoral and cellular factors. Since there is no proof yet that antibodies are involved with cellular or humoral activity, it remains to be investigated whether the active factor or factors associated with cells and in serum have a common origin or even are identical.

The cellular alterations responsible for the observed protection afforded by cell transfers are unknown and can only be conjectured. Numerous observations indicate that cells from infected animals differ from normal ones in many activities. There is no evidence that an antibody or antibodies might be responsible for these changes. The transfer factor released upon incubation of polymorphonuclear leukocytes of tuberculin-sensitive people with PPD has not been identified as being an antibody (20). There is, however, good reason to assume that cell-dependent, delayed hypersensitivity represents a first phase of the events leading to formation of antibody (21). This possibility and the fact that an alpha-globulin was found in cells of sensitized animals make further search for cell-bound antibody or antibody-like substance necessary. Successful transfer of delayed hypersensitivity with an alpha-globulin fraction from hyperimmunized guinea pigs, which were skin-tested with OT two days before the plasma was obtained, supports this argument (22).

Mononuclear phagocytes from tuberculous or BCG-infected animals exhibit some metabolic alterations compared with normal cells. Thus, elevated respiration and acid-phosphatase content have been demonstrated (16, 23). It appears that these changes are nonspecific as far as their elicitation is concerned and are an expression of heightened responsiveness of the phagocytic system. It is important to note that macrophages of some part of the body contain relatively large amounts of lysozyme, especially in the lung, and less in others (24). Accumulation of these cells, possibly with an induced elevated content of this enzyme, could contribute greatly to the formation of a local environment inimical to the tubercle bacillus. It has been suggested that immunization or infection act as selective mechanisms for more active and more resistant phagocytes (25). Data presented in this paper or elsewhere do not permit a decision as to whether such selective mechanisms are actually involved or whether the activities measured are subject to inductive variation. Delayed hypersensitivity might play an important role in mobilizing altered cells at the site of infection. Transfer of cells from sensitive donors should result in delayed responsiveness of the recipient. Whether this was actually the case was not tested.

A beneficial effect of local accumulation of phagocytes, either actively or passively, on subsequent infection at the same site with pathogenic organisms has been demonstrated (26, 27). It is unlikely that the success of the transfer experiments described is based on a similar local phenomenon. The test site in the former experiments coincided with the site of accumulation of cells, whereas it was distant from it in the present experiments. The possibility that some attenuated tubercle bacilli are transferred from the donor to the recipient with the cells has to be considered. The cultivation of transfer cells from peritoneal exudates occasionally yielded a few colonies, indicating that a small number of bacilli was transferred with the cells. It remains to be explored whether these organisms could induce active immunity in the recipients within the few days prior to challenge infection.

Experiments with brucellae and mycobacteria indicate that there is some cross immunity between these infectious agents (5). This might represent an increased capacity of mobilization of cellular elements with elevated activity. The mobilization could be triggered either by specific or nonspecific mechanisms. In addition, lymphatic hyperplasia as a consequence of primary infection has been shown to influence the progress of homologous or heterologous secondary infection (28). These and other observations on the influence of nonspecific stimuli on resistance to tuberculosis suggest that acquired immunity to tuberculosis is mediated at least in part by mechanisms which are independent of the antibody-forming system.

Summary

Some degree of protection against challenge infection with tubercle bacilli was achieved in guinea pigs and mice by the transfer of cells from BCG-vaccinated donors to normal recipients.

The effectiveness of cell transfer was measured by *in vitro* and *in vivo* techniques, namely, intracellular growth of tubercle bacilli within monocytes derived from recipients and the course of a challenge infection in cell recipients. In general, recipients of cells from BCG-infected donors exhibited some degree of inhibition of multiplication of bacilli used for challenge infection. The degree of inhibition was less than that achieved by BCG vaccination.

The protective effect was limited to challenge infection of an attenuated nature, that is, infection with either relatively large doses of attenuated tubercle bacilli or small doses of virulent bacilli. When the recipients were infected with large doses of virulent bacilli, their fate was no different from that of similarly infected controls.

Sumario

Traspaso Celular de la Inmunidad a la Tuberculosis Traspaso Pasivo de la Resistencia Adquirida a la Infección por el Mycobacterium Tuberculosis por Medio de Células

En cobayos y ratones se obtuvo alguna protección contra la infección provocativa con bacilos tuberculosos por el traspaso de células de donantes vacunados con BCG a receptores normales.

La eficacia del traspaso de células se midió con técnicas *in vitro* e *in vivo*, o sean, la proliferación intracelular de los bacilos tuberculosos dentro de monocitos derivados de los receptores y la evolución de una infección provocadora en receptores de células. En general, los receptores de células procedentes de donantes infectados con BCG manifestaron alguna inhibición de la multiplicación de los bacilos usados para la infección provocativa. La intensidad de la inhibición fué menor que la obtenida por la vacunación con BCG.

El efecto protector se limitó a una infección provocadora de naturaleza atenuada, es decir, infección ya con dosis relativamente grandes de bacilos tuberculosos atenuados o con dosis pequeñas de bacilos virulentos. Cuando se infectó a los receptores con grandes dosis de bacilos virulentos, su destino no fué distinto del de los testigos infectados de modo semejante.

Resume

Transfert cellulaire d'immunité à la tuberculose. Transport passif de résistance acquise à l'infection avec Mycobacterium tuberculosis au moyen de cellules

On parvint à obtenir quelque degré de protection contre l'attaque infectieuse de bacilles tuberculeux, chez des cobayes et des souris, par le transport de cellules de donneurs vaccinés par le BCG à des sujets normaux.

On mesura l'efficacité du transport cellulaire par des techniques *in vitro* et *in vivo*, précisément le développement intracellulaire de bacilles tuberculeux dans les monocytes obtenus chez les sujets, et l'évolution de l'attaque infectieuse dans les cellules des sujets. En général, les sugets qui reçurent des cellules de donneurs infectés par le BCG montrèrent quelque degré d'inhibition de la multiplication des bacilles utilisés pour l'attaque infectieuse. Le degré d'inhibition fut moindre que celui obtenu par la vaccination par le BCG.

L'effet protecteur fut limité pour lutter contre une infection atténuée, à savoir une infection soit avec des doses relativement fortes de bacilles tuberculeux atténués ou des petites doses de bacilles virulents. Quand les sujets furent infectés avec des fortes doses de bacilles virulents, leur sort ne fut pas différent de celui des contrôles infectés de la même façon.

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CALCIFIED TRACHEOBRONCHIAL LYMPH NODES IN DIFFERENT GENERATIONS[1]

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INTRODUCTION

The finding of calcified foci in the tracheobronchial lymph nodes is usually taken as evidence of tuberculous infection which occurred during the infancy or childhood of the person.[2] In the nineteenth century, childhood exposure to tubercle bacilli was almost universal. During the present century, however, the hazard of such exposure has diminished greatly in the United States. It seems possible that this decreased childhood exposure might be reflected in a decreased incidence of calcified tracheobronchial nodes found at post-mortem examination of tuberculous patients who were born during the more recent decades or, conversely, that patients born during the past forty years might show a higher incidence of primary adult tuberculous infection than those born during the nineteenth century. In an attempt to gather data on this subject, post-mortem roentgenograms have been made of the lungs of each patient who died and was examined at autopsy at the William H. Maybury Sanatorium during the past ten years. The present study summarizes the data thus obtained.

MATERIALS AND METHODS

During the interval from February, 1951, to June, 1960, the lungs and lower portion of the trachea of autopsied patients were removed and cared for in a manner so as to make the organs available for roentgenograms showing the tracheobronchial lymph nodes. After formalin fixation, a frontal slab from 2 to 3 cm. in thickness was removed from the middle portion of the right and left lungs, taking care to include the hilum and associated lymph nodes from each lung as well as several centimeters of the lower portion of the trachea. Roentgenograms of such tissue slabs exclude most of the lung parenchyma, but are quite adequate for demonstrating calcium in the tracheobronchial lymph nodes (figures 1 and 2). Such roentgenograms might be called post-mortem tomograms.

Patients are sent to the Maybury Sanatorium because of proved or suspected tuberculosis. In order to establish the diagnosis, each newly admitted patient is given a Mantoux test, his sputum is examined for tubercle bacilli by microscopy and culture and, if indicated, the gastric aspirate is cultured. The present study includes only those patients who had active tuberculosis at the time of death or who had had tuberculosis at some time, as indicated by sputum positive for tubercle bacilli, by a positive tuberculin reaction, and/or by the post-mortem findings. Three cases have been excluded because the laboratory and post-mortem findings indicated that the cause of death was lung abscess without concomitant tuberculous infection. Two other cases, one of histoplasmosis and one of carcinoma of the lung without associated tuberculosis, have been excluded. The women's unit of the Sanatorium was discontinued in 1955. For the sake of uniformity, the few specimens of women's lungs have been excluded also. This leaves for analysis the post-mortem tomograms of the lungs of 217 male patients who were twenty-three to eighty-eight years of age at the time of death, and similar tomograms of the lungs of 5 infants or children who were ten months to six years old at the time of death (figures 3 and 4).

In 2 cases, a white male born in Canada in 1877, and a Negro born in South Carolina in 1908, the tracheobronchial nodes did not show calcification, but there were calcified mesenteric nodes. It was assumed that these old mesenteric lesions also were the result of childhood infection with tubercle bacilli (1).

In tabulating the results, each patient has been assigned to the decade in which he was born. For example, those born in the years 1885 to 1894, inclusive, have been assigned to the 1890 decade; those born from 1895 to 1904, to the 1900 decade. Patients born during a given ten-year period will be referred to hereafter as a "generation" (2). Since the study covers Sanatorium deaths during a ten-year period, there is a considerable spread in the actual age at time of death of patients in a given generation. Thus, patients aged 47 to 56 were assigned to the 1900 generation if death occurred in 1951 but, if death occurred in 1960, patients in the 1900 generation were then 56 to 65 years of age. This makes a total age spread of 47 to 65 years for the 1900 generation at the time of death (table 1).

[1] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

[2] Although lymph node calcification may also be caused by histoplasmosis, the finding of calcified nodes in a patient with tuberculous infection usually justifies the assumption that *M. tuberculosis* was the underlying cause of the calcification.

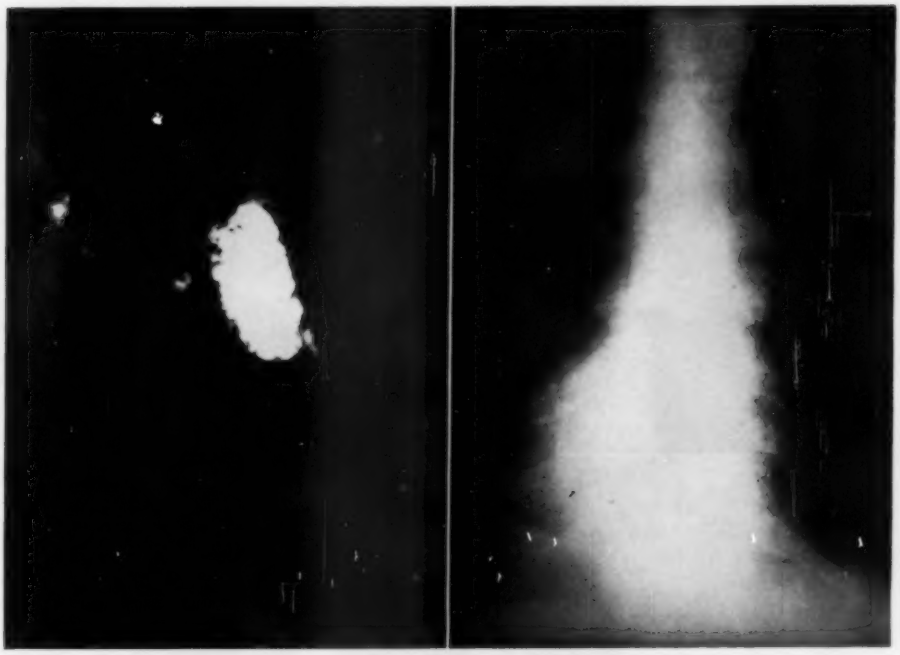

FIG. 1 (Left). A. 1162. Post-mortem tomogram showing large calcified node just below the bifurcation of the trachea.

FIG. 2 (Right). Ante-mortem film, same case. Being directly over the vertebrae, the calcified node is obscured.

OBSERVATIONS

The post-mortem findings with regard to calcified lymph nodes in 222 patients born during a 100-year interval are presented in table 1. The total number of cases in each generation is given, along with the number showing calcification in the lymph nodes. When a sufficient number of cases is involved, the percentage showing calcification is indicated. In the final column, the table gives the number and percentage of cases in which no calcified nodes were found. Presumably, these patients were infected as adults.

A progressive increase in the proportion of cases in which tubercle bacilli were demonstrated, either in the sputum or in the post-mortem sections, may be seen in table 2. This increase in positive reactions in the more recent generations is a reflection of the more acute type of tuberculosis which characterizes fatal infection in younger persons.

Among the 222 cases, a total of 155 showed calcium in the lymph nodes. In 75 per cent of the 155 patients, acid-fast bacilli were demonstrated in the sputum or in the post-mortem sections. Acid-fast bacilli were demonstrated in 70 per cent of the 67 cases which did not show calcified lymph nodes. Five patients, from fifty-six to eighty-one years of age, failed completely to react to tuberculin, including 10 mg. of OT. Nevertheless, all of these patients showed acid-fast bacilli in direct microscopy of the sputum (3), and all had calcium deposits in the tracheobronchial lymph nodes.

Included in the study is material from many patients who were born in Europe or the British Isles. The material from these patients was compared with material from patients born in the state of Michigan. When reduced to similar age groups (generations prior to 1910), there was no significant difference in the incidence of calcified lymph nodes (table 3).

When the material from adults was classified

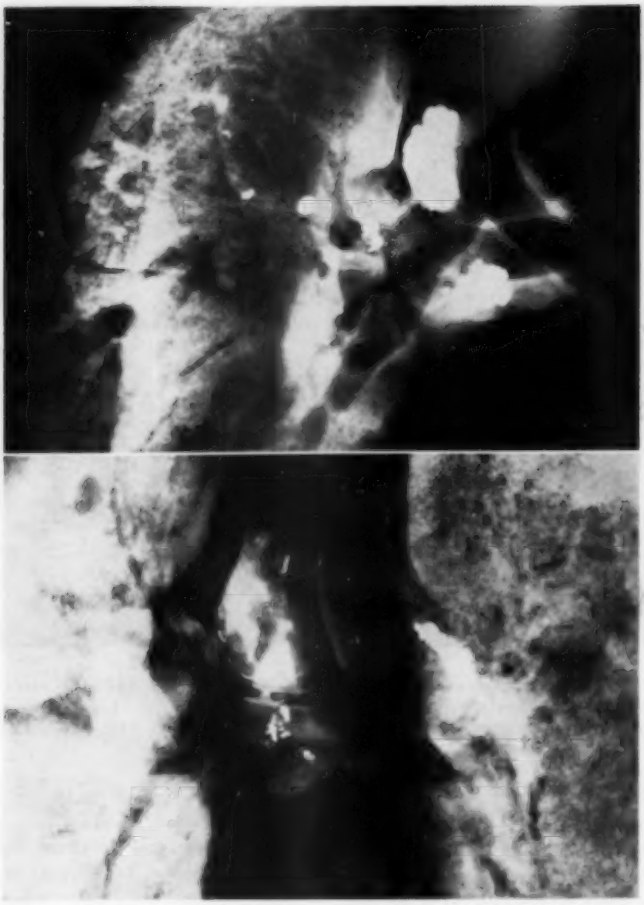

FIG. 3 (Top). A. 1332. Post-mortem tomogram showing right lung, bifurcation of the trachea, and calcified tracheobronchial nodes. In this case, also, the large node failed to show in the ante-mortem film.

FIG. 4 (Bottom). A. 1352. Post-mortem tomogram showing eggshell calcification in large node overlying the bifurcation. There are other calcified foci also. The patient, born in Ireland in 1898, was exposed to silica while working on the Lincoln Tunnel in New York City.

on the basis of its origin from Negro and non-Negro patients, no difference was noted in the incidence of calcified lymph nodes.

DISCUSSION

Just as the tombstones of a country graveyard may give clues to the local history, so calcified foci in the human body may give some insight into the epidemiologic problems of past decades. To properly interpret these relics of a bygone era it is essential that information be gathered while the script is still legible. In the present study, for example, only one patient is included from the 1860 generation.

The marked predilection for involvement of the lymph nodes in childhood tuberculosis was demonstrated even before the discovery of the tubercle bacillus (4). In addition, the greater tendency of the caseous lesions of children to calcify than those of adults has long been

known (5). More recently, Adler (6) has pointed out that, "The frequency with which calcification develops in pulmonary tuberculosis varies directly with the age at which the lesions begin. In the youngest age group calcifications develop in nearly 80%, whereas the rate declines in older children and decreases to a minimal percentage in cases where the disease commenced between the ages of 15 and 19." Thus, the assumption that calcified tracheobronchial nodes in an adult tuberculous patient mean primary infection in childhood seems justified. The further assumption that *absence* of calcified lymph nodes in an adult tuberculous patient indicates primary infection during adult life is supported in many of the cases by a definite history of exposure to tubercle bacilli during adult life.

Post-mortem study of the lungs by means of roentgenograms was introduced by Opie (7). The method has been used extensively by Terplan (8) and Sweaney (9). An exhaustive roentgenographic study of post-mortem lungs has been made by Birkelo and Brosius (10). It is unfortunate that, although the present study represents a ten-year effort, the number of cases is so few.

A fairly constant incidence of calcified nodes in the 1870, 1880, and 1890 generations, the probable adult infection in those years varying from only 21 to 25 per cent, may be seen in table 1. In the 1900 and 1910 generations, the proportion showing calcified nodes has decreased and, conversely, the proportion of cases probably infected as adults has increased rather abruptly.

The possibility of decreased childhood exposure seems inadequate to explain the increased adult infection in the 1900 generation. Actually, during the period involved (1895–1904), there was no general knowledge of Koch's work,[3] little or no pasteurization of milk, and no attempt to control bovine tuberculosis. The more probable explanation lies in the increased *exposure* to tuberculosis of the young adults of this generation. It was largely the 1900 generation, young men twenty-two years of age and less in 1917, who fought in the first World War. The hazard of tuberculous infection experienced by those men is well known (11).

In the 1910 generation, which has the highest indicated adult infection rate, a factor of lessened childhood exposure might be involved. However, the young men of this generation also experienced an increased hazard of adult exposure, as members of the Civilian Conservation

[3] The National Association for the Study and Prevention of Tuberculosis was organized in June, 1904.

TABLE 1

CALCIFIED LYMPH NODES IN DIFFERENT GENERATIONS

Generation	Age at Death	Number of Cases	Calcification in Nodes		No Calcification (Infected as Adults?)	
			Number	Per Cent	Number	Per Cent
1860	87–105	1	1			
1870	77–95	12	9	75	3	25
1880	67–85	43	33	77	10	23
1890	57–75	72	57	79	15	21
1900	47–65	50	32	64	18	36
1910	37–55	30	18	60	12	40
1920	27–45	7	5	71	2	29
1930	17–35	2	1			
1940	7–25	0				
1950	<15	4	1			
1960	<5	1	0			

TABLE 2

PATIENTS WITH DEMONSTRABLE TUBERCLE BACILLI

Generation	Total Number of Cases	Tubercle Bacilli Demonstrated	
		Cases	Per Cent
1860	1	1	
1870	12	5	42
1880	43	29	67
1890	72	49	68
1900	50	37	74
1910	30	27	90
1920	7	7	100
1930	2	2	
1940	0	0	
1950	4	4	
1960	1	1	

TABLE 3

COMPARATIVE INCIDENCE OF CALCIFIED NODES IN FOREIGN-BORN AND MICHIGAN-BORN PATIENTS
(Generations 1860–1900 Inclusive)

Place of Origin	Number of Cases	Calcified Nodes	Per Cent
Patients born in Europe	58	41	71
Patients born in Michigan	33	24	73

Corps during the early 1930's and as soldiers during World War II.

The figure of 29 per cent for the adult infection rate in the 1920 generation is based on too few cases to be given much consideration. The definitely lessened childhood exposure to tubercle bacilli experienced by this generation should have resulted in a *higher* adult infection rate. However, in this age group the factor of chemotherapy undoubtedly played an important role. In all of the period during which autopsy material for the present study was being gathered (1951–1960), streptomycin and PAS were available; isoniazid was available during the latter part. Experience has shown that any recent tuberculous infection in relatively young persons responds well to today's potent chemotherapies, and the Maybury Sanatorium records contain many instances of the lifesaving effect of chemotherapy for young men of the 1920 generation (12). The lives of patients in the other generations also have been saved by chemotherapy. Particularly in the 1920 and 1930 generations, however, the deaths at Maybury (and the number of autopsies) were greatly decreased by chemotherapy, and this was particularly true of the patients "probably infected as adults."

No representative of the 1940 generation was examined at autopsy during the period of observation, for the Sanatorium has relatively few patients in this age group. Moreover, this is the group whose disease was most favorably influenced by chemotherapy.

In spite of the near miracles accomplished by chemotherapy, the occasional impossible cases remain. The 1950 and 1960 generations are represented by 5 young patients who were admitted with disease so far advanced that their condition was hopeless.

Table 2, in which a progressive increase may be seen in the proportion of patients with demonstrable tubercle bacilli in the sputum, is suggestive of the different types of disease found in the representatives of the different generations. Patients from the 1870 generation were mostly "good chronics." Most of them died from cerebral or cardiovascular disease. All of the 1920 generation, on the other hand, had active tuberculosis; all of them who had autopsy examinations died from progression of their tuberculous disease.

It is indicated in table 3 that conditions with regard to childhood exposure to tubercle bacilli were neither better nor worse in Michigan than they were in Europe during the period from 1860 to 1900.

Summary

In order to demonstrate calcified tracheobronchial nodes, roentgenograms have been made of 2- to 3-cm. slabs of fixed lung tissue, including the hilum and lower portion of the trachea. The resulting data, gathered from 222 autopsies, have been tabulated according to the decade in which the patient was born. The incidence of calcified nodes and, conversely, of probable adult infection, is fairly constant for 1870, 1880, and 1890. An increase in incidence of adult infection for the 1900 and 1910 generations is indicated. This is believed to be due to excessive exposure to tubercle bacilli during adult life rather than to lessened incidence of childhood tuberculosis.

Contrary to expectation, the data show a *smaller* proportion of deaths from primary adult tuberculosis in the 1920 generation than in the two preceding generations. The excellent effect of chemotherapy on recent infections in young adults is offered as an explanation of this finding.

In older patients the incidence of calcified tracheobronchial nodes was found to be almost identical in persons born in Europe and those born in Michigan.

Sumario

Calcificación de los Ganglios Linfáticos Tráqueobronquiales en Distintas Generaciones

A fin de descubrir los ganglios tráqueobronquiales calcificados, se tomaron radiografías de tajadas de 2 a 3 cm. de tejido pulmonar fijado, comprendiendo el hilio y la porción inferior de la tráquea. Los datos resultantes, acopiados de 222 autopsias, han sido tabulados de acuerdo con el decenio en que había nacido el enfermo. La incidencia de la calcificación ganglionar, y recíprocamente, de probable infección adulta, es bastante constante para 1870, 1880 y 1890, hallándose indicado un aumento en la incidencia de infección adulta para las generaciones de 1900 y 1910. Esto parece deberse a excesiva exposición a los bacilos tuberculosos durante la vida adulta más bien que a menor incidencia de tuberculosis infantil.

En contraposición a lo esperado, los datos muestran una proporción *menor* de muertes debidas a tuberculosis adulta primaria en la generación de 1920 que en las dos generaciones precedentes. Como explicación de este hallazgo se ofrece el

excelente efecto de la quimioterapia en las infecciones recientes en adultos jóvenes.

En los enfermos más viejos, la incidencia de ganglios tráqueobronquiales calcificados resultó ser casi idéntica en las personas nacidas en Europa y las nacidas en Michigan.

Resume

Ganglions lymphatiques trachéobronchiques calcifiés de différentes générations

Afin de prouver l'existence de ganglions trachéobronchiques calcifiés, les auteurs ont radiographié des tranches de 2 à 3 cm de tissu pulmonaire fixé, comprenant le hile et la partie inférieure de la trachée. Les données qui en résultèrent, recueillies à partir de 222 autopsies, ont été classées selon la décade de la naissance du malade. La fréquence de nodules calcifiés et, par la même, de l'infection probable de l'adulte, est à peu prés constante pour 1870, 1880 et 1890. Une augmentation de la fréquence de l'infection de l'adulte fut indiquée pour 1900 et 1910. On croit que ceci est dû à un contact excessif avec le bacille tuberculeux pendant la vie adulte, plutôt qu'une fréquence moins grande de la tuberculose de l'enfance.

Contrairement à ce que l'on attendait, les données montrent, dans la tuberculose pulmonaire de l'adulte, une proportion de morts *plus petite* pour 1920 que pour les deux générations précédentes. L'effet excellent de la chimiothérapie sur les infections récentes des jeunes adultes est offerte comme explication de cette découverte.

On a trouvé, chez des malades plus agés, une fréquence de ganglions trachéobronchiques calcifiés presqu'identique chez des personnes nées en Europe et chez celles nées dans le Michigan.

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THE PERSISTENCE OF TUBERCLE BACILLI IN CASEOUS LESIONS IN THE EXPERIMENTAL ANIMAL (GUINEA PIG)[1, 2]

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Introduction

Many caseous lesions resected from the lungs of tuberculous patients who have received antimicrobial therapy still contain morphologically intact tubercle bacilli which may be nonculturable as well as nonpathogenic for laboratory animals. Moreover, these bacillary cells are sometimes observed in very large numbers in stained preparations (1). A number of investigations have been undertaken to establish the viability of such cells, using both conventional and modified culture techniques (2–6); inoculation into a susceptible animal host; and the use of silica (7, 8), steroids, and X-ray (9) in order to increase host susceptibility.

The results of these studies indicate that no single culture technique or combination of culture and animal inoculation techniques has been capable of isolating tubercle bacilli from some caseous lesions, even though they contained myriads of stainable organisms.

The present studies were designed to determine how long heat-killed tubercle bacilli will remain morphologically intact and stainable in caseous lesions in the guinea pig, and also to discover how long living, avirulent tubercle bacilli, under similar conditions, will remain stainable and culturable by conventional techniques.

The living avirulent H37Ra strain was used because it would produce local nonprogressive caseous lesions and the animals could be followed for long periods of time. Living virulent H37Rv organisms were not used because they produce local caseous lesions and disseminated progressive disease, causing the animals to succumb to their disease within sixty to ninety days unless they receive antimicrobial therapy. With such treatment the local caseous lesions usually disappear within a year.

[1] From the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported in part by research grants (E36C8 to E36C13, inclusive) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

Studies on the survival of bovine tubercle bacilli in caseous pulmonary lesions in rabbits that had received antimicrobial therapy have been reported elsewhere (10–15).

This report is divided into two parts, Study I and Study II.

Observations

Study I

A pellicle of a three-week-old culture of living H37Rv tubercle bacilli from modified Proskauer and Beck fluid synthetic medium was triturated in a Pyrex tube with the aid of a Teflon grinder. Physiologic saline was added drop by drop during the grinding process until the suspension was milky in appearance. The suspension was transferred to a glass vial, sealed by flame, placed in a water bath, brought to the boiling point, and maintained there for an hour and a half. A stained smear of the suspension at that time showed large numbers of single and clumped organisms.

A similar growth of a three-week-old culture of living avirulent H37Ra tubercle bacilli from modified Proskauer and Beck fluid synthetic medium was prepared as described above but not killed.

Eighty-four guinea pigs, weighing approximately 300 to 400 gm. each, half of which were males and half females, were divided into two groups of 42 animals each. The 21 males of the first group were inoculated with 0.6 ml. of the prepared suspension of H37Rv heat-killed tubercle bacilli as follows: 0.2 ml. in the right testicle, 0.2 ml. in the region of the right groin, and 0.2 ml. intraperitoneally. The 21 females in this group were inoculated with the same suspension, but received only 0.2 ml. in the right groin and 0.2 ml. intraperitoneally.

The remaining group of 42 guinea pigs of similar sex and weight was divided as above and inoculated with the prepared living avirulent H37Ra tubercle bacilli.

All animals were tested intracutaneously with 0.1 ml. of Old Tuberculin (OT) one month after inoculation. They all responded with good skin reactions. As a rule, those that had received the living H37Ra organisms responded more strongly than those that had received the heat-killed H37Rv tubercle bacilli.

Three animals in each group, i.e., the pigs that had received heat-killed H37Rv and those that had received living H37Ra organisms, were sacrificed at two-month intervals. A balance between the number of males and females surviving

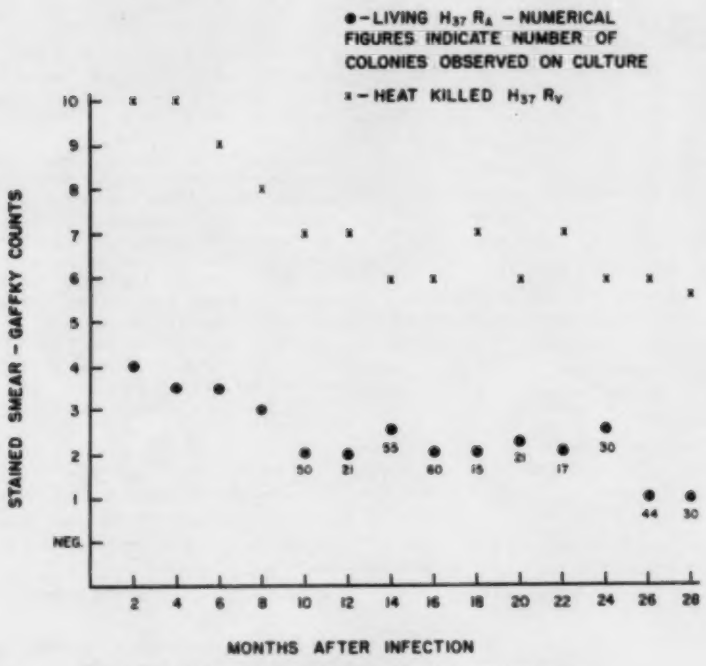

FIG. 1. Tubercle bacilli in necrotic lesions (Study I).

throughout the experiment was attempted. In other words, if an animal in one group died spontaneously of an intercurrent infection or because of some unknown cause, an animal in the opposite group was sacrificed. The bacteriologic findings from the interim deaths were averaged with those at the closest sacrifice period. All animals were skin-tested with 5 per cent OT prior to being killed. Autopsy examination generally revealed caseous lesions which usually appeared at the sites of inoculation, i.e., the inguinal and testicular regions and in the omentum in the peritoneum. A small amount of this caseous material was removed from each lesion, smeared on a slide, and stained by the Ziehl-Neelsen technique. The smears were examined microscopically and the Gaffky scale[3] was used for recording the number of stainable organisms observed.

The caseous material removed from the animals that had received the living H37Ra tubercle bacilli was stained and likewise examined microscopically. It was emulsified in a small amount of physiologic saline and cultured directly on American Trudeau Society medium. Growth was recorded as follows: 4-plus for confluent growth, a gradual grading down to 3-, 2-, and 1-plus, depending upon the relative amount of growth as compared with a 4-plus growth. From 0–100, colonies were recorded numerically (figure 1).

It may be noted in figure 1 that at the first sacrifice period the average bacterial count in stained smears of the caseous contents of the lesions from animals that had received heat-killed H37Rv tubercle bacilli was Gaffky X, whereas the average bacterial count in the material removed from the animals that had received the living H37Ra organism was Gaffky IV.

The marked difference between the bacterial counts of the two groups at that time may have been more apparent than real, since the suspensions were not adjusted to contain approximately the same number of tubercle bacilli before injection into the animals.

From the first sacrifice period on, there was a

[3] *Gaffky scale:* I: Only 1–4 bacilli in the whole preparation; II: Only 1 bacillus on average in many fields; III: Only 1 bacillus on average in each field; IV: 2–3 bacilli on average in each field; V: 4–6 bacilli on average in each field: VI: 7–17 bacilli on average in each field; VII: 18–25 bacilli on average in each field; VIII: 50 bacilli on average in many fields; IX: 100 bacilli on average in many fields; X: Enormous masses on average in many fields.

gradual decrease in the bacillary content of the caseous lesions from both groups of animals. This decrease continued to the tenth month, when a plateau was reached which remained at approximately the same level to the sacrifice period at the twenty-eighth month, at which time the experiment was terminated.

The number of H37Ra organisms recoverable by culture gradually decreased from a 2- to 3-plus growth at the first sacrifice period to a 1-plus at the sacrifice period in the eighth month, and to numerical growth from the tenth to the twenty-eighth months. During this period the growth ranged from a low of 16 colonies to a high of 60, or a mean of 34 colonies per sacrifice period from the tenth to the twenty-eighth months, inclusive.

As stated, stained smears to note the bacillary content of the suspensions prior to inoculation into animals had not been made, nor had the same variant of the H37 strain of tubercle bacilli been used in preparing the heat-killed and living suspensions. Therefore, with these omissions in mind, another study was undertaken.

Study II

The procedures followed in Study I were more or less duplicated in Study II. The living and heat-killed suspensions were prepared from the same culture of H37Ra tubercle bacilli, and at this time were adjusted to contain approximately an equal number of organisms per milliliter. When possible, the same amount (double loopful) of caseous material was removed from each animal at autopsy for microscopic study and culture. The duration of the experiment was thirty-six months.

One hundred and eight guinea pigs, each weighing 350 to 400 gm., half males and half females, were used. They were divided equally as to sex and weight and were given 0.2 ml. of the suspension of living or dead tubercle bacilli at each site of inoculation, as the animals in Study I had received. Similarly, 3 pigs from each group were killed at the various sacrifice periods for comparison.

In figure 2 it will be noted that *initially*, prior to inoculation in animals, both suspensions contained approximately the same number of tubercle bacilli. Also, as in Study I (figure 1), there was a gradual decrease in the number of stainable organisms in the caseous lesions from

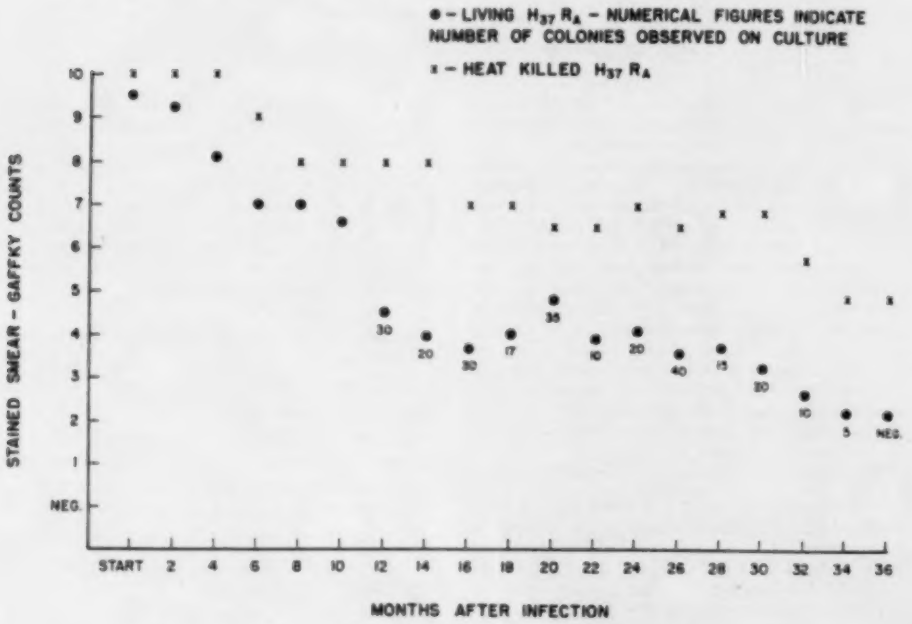

FIG. 2. Tubercle bacilli in necrotic lesions (Study II).

the guinea pigs that had received the heat-killed and the living avirulent tubercle bacilli. Likewise, there was a definite drop in the number of organisms recoverable by culture from the lesions of the animals that had received living H37Ra tubercle bacilli.

Discussion

When heat-killed tubercle bacilli from exponentially growing cultures were inoculated into guinea pigs by various parenteral routes, they produced caseous lesions and, within the lesions, the bacilli persisted and remained stainable in decreasing numbers for up to thirty-six months.

Living tubercle bacilli of the H37Ra strain likewise remained stainable in the caseous lesions for up to thirty-six months, except that they were culturable only up to the thirty-fourth month. At some of the sacrifice periods, a few marked differences were observed in the bacillary content of some of the lesions of the animals that had received heat-killed, and of those animals that had received living, organisms. These differences were compensated for, however, by using the average findings in 3 pigs at each sacrifice period. There was a more pronounced drop in the number of stainable organisms in the lesions of the animals that had received living organisms than in the lesions of the animals that had received dead organisms. A possible explanation for this difference could be that heat-killing changed the physical and chemical state of the organisms and destroyed the intracellular enzymes which, under normal conditions, would bring about a more rapid destruction of the organisms. Therefore, it is reasonable to assume that certain enzymes and chemical constituents (16, 17) of the caseous lesions may not be so destructive for artificially killed organisms as for those whose deaths occur from ordinary *in vivo* local environmental effects.

The number of stainable and culturable organisms present in the caseous lesions at the sacrifice period in the thirty-sixth month was figured by rough calculation. There were approximately 600,000 oil immersion fields to the average smear preparation examined. A well-trained microscopist covered about 1,200 fields in twenty minutes, which was the time devoted to scanning each preparation. The average bacillary content of the smears from lesions of the animals that had received the heat-killed organisms was Gaffky V, or 4 to 6 organisms in each microscopic field. Using the above figures for the calculations, this is equivalent to approximately three million tubercle bacilli for the slide preparation. The caseous lesions as a whole would contain a great many times this number. The smears from the lesions of the animals that had received the living organisms (H37Ra) had Gaffky II counts, or one organism in ten fields, the equivalent of 60,000 tubercle bacilli to the smear preparation. Even with such large numbers of residual organisms present in the lesions and the fact that the animals received no antimicrobial therapy, it was impossible to isolate organisms by ordinary, well-accepted culture techniques.

Summary

In these studies it has been shown that heat-killed H37Rv and H37Ra tubercle bacilli persisted and retained their staining properties, although in decreasing numbers, in caseous lesions in guinea pigs up to twenty-eight and thirty-six months, respectively.

Living avirulent H37Ra tubercle bacilli likewise remained stainable in decreasing numbers for the same length of time, and culturable up to thirty-four months.

All animals retained their sensitivity to tuberculin. Most of the animals, however, had markedly diminished skin sensitivity to tuberculin by the thirty-sixth month, when the experiment was terminated.

Sumario

La Persistencia de los Bacilos Tuberculosos en las Lesiones Caseosas del Animal de Experimentación (Cobayo)

En estos estudios se ha demostrado que los bacilos tuberculosos H37Rv y H37Ra matados al calor persistían y retenían sus propiedades de coloración, aunque en números menores, en las lesiones caseosas de los cobayos hasta veintiocho y treinta y seis meses, respectivamente.

Los bacilos tuberculosos H37Ra avirulentos vivos también continuaban siendo colorables en números decrecientes durante el mismo período de tiempo, y culturables hasta treinta y cuatro meses.

Todos los animales retuvieron su sensibilidad a la TA. Sin embargo la mayoría de ellos mostraban notable disminución de la cutisensibilidad para el trigésimo-cuarto mes, cuando se terminó el tratamiento.

Resume

La persistance de bacilles tuberculeux dans des lésions caséeuses chez l'animal d'expérience (cobaye)

Dans ces etudes, l'auteur a montré que des bacilles tuberculeux H37Rv et H37Ra, tués par la chaleur, survivaient et gardaient leurs propriétés de coloration, bien qu'en nombres décrus, dans des lésions caséeuses de cobayes, aussi longtemps que vingt-huit et trente-six mois pour chacun d'eux.

Des bacilles tuberculeux H37Ra vivants et non virulents restèrent colorables de la même façon en nombres réduits, pendant la même durée et furent cultivables jusqu'à trente-quatre mois.

Tous les animaux gardèrent leur sensibilité à OT. La plupart des animaux, cependant, eurent une sensibilité cutanée nettement diminuée vers le trentesixième mois, quand l'expérience prit fin.

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Case Report

BRONCHOGENIC CARCINOMA WITH CUSHING'S SYNDROME[1]

A Review with the Report of an Additional Case

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(Received for publication October 3, 1960)

Introduction

In 1952, Thorne (1) reported two cases of Cushing's syndrome with bronchogenic carcinoma of the oat cell type. He reviewed the world's literature on this particular combination and the more general one of Cushing's syndrome and neoplasia (2–14). Since his report, twelve additional cases of Cushing's syndrome associated with oat cell bronchogenic carcinoma have been published (15–25), and publication is pending on several others (26–29). In addition, two cases associated with bronchogenic adenocarcinoma (30, 31), a single case associated with prostatic adenocarcinoma (32), and a case associated with breast carcinoma (21) have been reported. The present paper presents a review of the literature, reports an additional case of Cushing's syndrome associated with oat cell carcinoma, and briefly mentions a second case with significant adrenal hyperplasia and oat cell carcinoma.

Case Report

J.H. (WHVA Hosp. #23257), a 74-year-old white male, was admitted to the West Haven Veterans Administration Hospital for the first time on January 31, 1960. He had had cardiac disease of unknown etiology, necessitating maintenance digitoxin during the preceding ten years. Six weeks prior to admission he developed severe dyspnea and orthopnea. Coincident with this, he noted the onset of cough productive of thick brown sputum. A chest roentgenogram obtained at another hospital one month prior to admission suggested "lung cancer." Two weeks later the diagnosis of "anaplastic carcinoma" was established by supraclavicular lymph node biopsy. The patient declined X-ray treatment. During hospitalization for the biopsy, the serum potassium was noted to be "slightly low." Meralluride (Mercuhydrin®), benzydroflumethiazide (Naturetin®), and aldactone were begun two weeks prior to admission. Two days preceding admission the patient became irritable and anoretic.

On admission to the hospital the patient was normotensive but stuporous. Coarse inspiratory and expiratory rhonchi were audible throughout both lung fields. He was treated for congestive heart failure with intermittent positive pressure breathing, rotated tourniquets, morphine sulfate, and aminophylline. Although the clinical signs of heart failure responded to therapy, the patient's sensorium did not clear and he survived only four days. Serum electrolytes and other pertinent laboratory data obtained during this period are listed in table 1. Electrocardiograms demonstrated ST and T wave changes that were compatible with hypokalemia and digitalis toxicity. Hypokalemia was at first attributed to the preceding diuretic therapy but, when correction proved difficult (table 1), excessive adrenocortical function was suspected. A single twenty-four-hour urine specimen contained markedly elevated steroids (table 1).

Autopsy findings (WHVA A-60-22): The patient was a well-developed, well-nourished, elderly male. Small pleural and abdominal effusions were present. The heart was enlarged. Coronary arteries were sclerotic but not occluded. A scar was present in the posterior wall of the left ventricle. The lungs were edematous. A large mass of confluent tumor nodules occupied most of the right lower lobe. Tumor had spread to the right hilar, paratracheal, and supraclavicular lymph nodes. A few small tumor nodules were found in the liver. No metastases were present in the adrenals, pituitary, or brain. The tumor was composed of small oval cells with scanty cytoplasm (figure 1). The adrenals were symmetrically enlarged: the combined weight was 29.9 gm. The cortex measured 2 to 3 mm. in thickness and was composed of large polygonal cells with granular eosinophilic cytoplasm (figure 2). Some cells had enlarged hyperchromatic nuclei. The pituitary was of normal size. Vacuoles were noted in pituitary basophils.

Comment: This case shows the characteristic diffuse adrenal hyperplasia (figure 2) with loss of zonation and large granular cortical cells described by one observer (32) as "reminiscent of the fetal X-zone." Hypokalemic alkalosis with elevation of urinary 17 hydroxysteroids and 17 ketosteroids were observed. Aldosterone was not measured. However, in one case associated with oat cell carcinoma (21) and in the case associated with prostatic carcinoma (32), al-

[1] From the Laboratory Service, Veterans Administration Hospital, West Haven, Connecticut.

TABLE 1

SERUM ELECTROLYTES AND OTHER PERTINENT LABORATORY DATA

Date	BUN*	Na†	K‡	CO2§	Cl‖	K intake
	mg/100 ml	mEq/L	mEq/L	mEq/L	mEq/L	mEq/24 hours
1/31/60	43	149	2.3	42	81	40
2/1/60	37	148	2.0	52	68	200
2/2/60	50	146	2.7	52	72	240
2/3/60	68	149	5.5	46	81	60

24-hour urine, Vol. 675 + c.c.
(mEq/L) Na 12 Cl 14 K 110
17 ketosteroids 68.5 mg.
17 ketogenic steroids 72 mg. } per 675 ml.

* Blood urea nitrogen.
† Sodium.
‡ Potassium.
§ Carbon dioxide.
‖ Chlorine.

dosterone concentrations in urine were elevated; while in three cases associated wtih bronchogenic carcinoma aldosterone concentrations in the urine were low (22, 23, 29).

DISCUSSION

The cases of Cushing's syndrome associated with neoplasia reported to date are summarized in table 2. These cases differ quantitatively from classic Cushing's syndrome in the following ways (1, 17, 23–25):

The progress of Cushing's syndrome is much more fulminant in those patients with coincident carcinoma. Death usually occurs from some complication of the adrenocortical hyperfunction, such as overwhelming sepsis or heart failure, within a few months of the onset of symptoms. By contrast, Plotz (33) found fifteen of thirty patients with untreated Cushing's syndrome not associated with carcinoma still alive nine years after diagnosis was established.

Hypokalemic alkalosis is a prominent symptom and frequently a presenting symptom in the cases of Cushing's syndrome associated with bronchogenic carcinoma, but is rarely seen in Cushing's syndrome not associated with carcinoma (33).

The sex ratio is reversed. The ratio was nine females to one male in Plotz's thirty cases of uncomplicated Cushing's syndrome, but he noted a ratio of three females to one male in a review of the literature (189 other cases) (33). Mason (34) found a four-to-one female-to-male ratio in a review of fifty-two cases. By contrast, four of the seventeen cases associated with oat cell carcinoma reported to date have occurred in females. This reversal is due almost entirely to cases associated with pulmonary carcinoma, a predominantly male neoplasm.

More patients are in an older age group, with an average age of fifty-two for those with carcinoma and thirty-one for those without (33).

The adrenals of the two groups are dissimilar with respect to weight and morphology.

Two recent studies discuss the diffuse adrenal cortical hyperplasia present in many cases of Cushing's syndrome not associated with carcinoma. Ashworth and Garvey (35) describe their findings in four such cases, while Cohen and associates (36) describe sixteen cases of bilateral adrenocortical hyperplasia. The former writer gives a weight range of 11–19 gm.; the latter, 10–26 gm. By contrast, in all but one of the cases associated with carcinoma, adrenal weights above 20 gm. were reported; the weights of the majority were greater than 30 gm.; and several were 50 gm. or more. The consistent morphologic features of the adrenal cortex in the cases not associated with carcinoma were increased thickness of the fasciculata-reticularis, large, relatively lipid-poor granular eosinophilic cells making up the inner fasciculata and entire reticularis (35, 36). Adrenals from the patients of cases associated with carcinoma also showed thickening of the fasciculata-reticularis, but the cells responsible for this were even larger than those in the non-cancer group, having a granular eosinophilic cytoplasm, hyperplastic hyperchromatic nuclei, and a uniform distribution from capsule to medulla.

In discussing possible interrelationships between Cushing's syndrome and carcinoma, Thorne (1) and more recent writers (20, 24, 25) have stressed the preponderance of a particular type of pulmonary carcinoma, the small cell undifferentiated type, and have tended to consider these cases apart from those associated with other types of cancer. Actually, with the exception of the single cases associated with adenocarcinoma of the prostate (32), intraductal carcinoma of the breast (21), and sym-

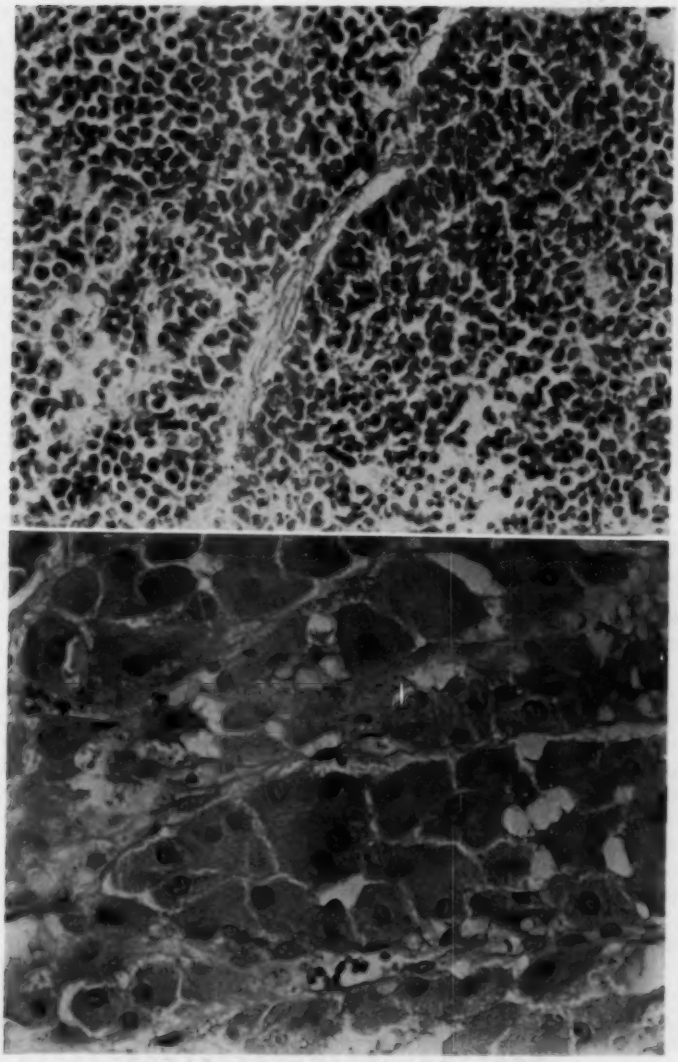

FIG. 1 (Top). Carcinoma—Case J. H. (×300)
FIG. 2 (Bottom). Adrenal cortex—Case J. H. (×300)

pathicoblastoma (37), the cases thus far reported fall into three histologic groups.

The most numerous group includes those cases associated with bronchogenic carcinoma (1, 2, 15–31). A second group includes four cases (3–6), and possibly a fifth (38), associated with neoplasms of pancreatic origin. Morphologic descriptions of several of these cases (3–5) suggest a possible islet cell origin. None of these pancreatic neoplasms are the common type of adenocarcinoma. The third group is made up of those cases associated with thymic tumors. This group includes five cases associated with small benign thymomas (3, 7, 8, 12, 13) and four classified as malignant thymomas (7, 9, 11, 14). The pathologic descriptions from three (7, 11, 14) of these

TABLE 2

CASES OF CUSHING'S SYNDROME ASSOCIATED WITH NEOPLASIA REPORTED TO DATE

Author	Year	Diagnosis	Age	Race	Sex	Duration	Adrenal Weight	Urinary Steroids			Hypokalemic Alkalosis	Diabetes	Cushinoid Appearance	Pituitary Metastases	Adrenal Metastases
								mg/24 hours		γ/24 hours					
								17 Ketosteroids	17 Hydroxysteroids	Aldosterone					
						months									
Brown	1928	Bronchogenic oat cell	45	W	F	—	—	—	—	—	+	—	+	0	0
Thorne	1952	Bronchogenic oat cell	36	W	M	6	—	24.3, 17.0	—	—	—	+	+	0	0
Thorne	1952	Bronchogenic oat cell	44	W	M	3.5	—	29.2	—	—	—	—	+	+	+
Castleman (CR-MGH)*	1953	Bronchogenic oat cell	76	W	F	—	38	43.2	—	—	+	0	0	+	0
White	1954	Bronchogenic oat cell	58	W	M	3	Increase	—	—	—	+	0	0	+	+
Spaulding	1955	Bronchogenic oat cell	56	W	M	2	34	11.3	8.1	—	+	+	0	—	+
Metzler	1955	Bronchogenic oat cell	46	W	M	3	25.5	22.0	71–90	—	—	+	—	+	+
Querido	1955	Bronchogenic oat cell	46	W	M	4	49.5	96, 60	—	—	+	+	0	+	+
Harrison	1957	Bronchogenic oat cell	55	W	F	8	Normal	5.6, 1.8	Normal	—	+	+	+	0	0
Castleman (CR-MGH)	1958	Bronchogenic oat cell	68	W	M	—	30	23.0, 17.0	39.0, 34.0	—	+	—	—	0	0
Kovach	1958	Bronchogenic oat cell	22	W	M	—	86	—	—	—	+	+	+	—	0
Lockwood	1958	Bronchogenic oat cell	61	W	M	2	50	18.5	92.8	24	+	+	—	—	+
Mach	1958	Bronchogenic oat cell	54	W	M	7	—	21.0	9.7	—	+	+	0	0	+
Mach	1958	Bronchogenic oat cell	60	W	F	1	23	—	37.0	1	+	0	0	0	+
Lewis	1960	Bronchogenic oat cell	41	N	M	11	65	55.0	113	—	+	+	+	+	+
Gelfman	1960	Bronchogenic oat cell	74	W	M	—	29.9	67.4	72.6	—	+	+	0	0	0
Bernanke	1960	Bronchogenic oat cell	53	W	M	4	40.0	35.0	41.5	4	+	+	0	0	0
Rosenthal	1957	Bronchogenic adenocarcinoma	48	W	M	2	30.0	50.4	138.0	—	0	+	0	0	+
MacPhee	1959	Bronchogenic adenocarcinoma	42	W	F	36	14.0	8.5	18.0	—	+	+	+	—	—
Webster	1959	Prostate	68	W	M	—	39.0	17.0, 34.0	34.0, 41.0	60	+	+	0	+	0
Lockwood	1958	Breast	53	W	F	24	44.0	24.0	—	—	+	+	+	0	0
Leyton	1931	Malignant thymoma	31	W	M	14	44.0	—	—	—	—	+	+	—	—
Frank	1945	Malignant thymoma	19	W	F	—	—	—	—	—	—	—	—	—	—
Dancot	1948	Malignant thymoma	40	W	M	13	30.0	—	—	—	—	0	+	—	—
Siegmund	1948	Malignant thymoma	46	W	M	—	Increase	—	—	—	?	?	+	0	0
Kepler	1933	Pancreas	46	W	F	4	—	—	—	—	—	0	+	—	—
McLetchie	1942	Pancreas	26	W	F	1.5	28.8	—	—	—	—	0	+	—	—
Mellgren	1945	Pancreas	50	W	F	12	—	—	—	—	—	0	+	—	—
Crooke	1946	Pancreas	28	W	F	3	24.9	15.0	—	—	—	+	+	—	—
Leyton	1931	Thymoma	11	W	M	—	20.6	—	—	—	—	—	—	—	—
Kepler	1933	Thymoma	46	W	F	—	—	—	—	—	—	—	—	—	—
Anderson	1944	Thymoma	34	W	F	—	—	—	—	—	—	—	—	—	—
Hubble	1949	Thymoma	—	—	—	—	—	—	—	—	—	—	—	—	—
Sprague	1950	Thymoma	15	W	M	24	49.0	Increase	Increase	—	+	+	+	—	—

* Case records, Massachusetts General Hospital. — No data available. 0 Negative finding. + Positive finding.

malignant cases strongly suggest bronchogenic carcinoma. It may be that the adrenal hyperfunction associated with these unusual pancreatic and benign thymic neoplasms represents an association distinct from that of the bronchogenic carcinoma group.

Although a few of the cases included in this review may simply represent the coincidental occurrence of Cushing's syndrome and neoplasia (13, 31), the majority undoubtedly represent a cause-and-effect relationship. The large number of cases now reported and their significant differences from classic Cushing's syndrome attest to this relationship. Some of the earlier writers (15, 17, 24) speculated that Cushing's syndrome might predispose to carcinoma. It now seems much more likely that the tumor precedes the adrenal hyperfunction (1, 23–25, 32). In favor of this is the reversed sex ratio due to the prevalence in the series of the predominantly male bronchogenic carcinoma, while Cushing's syndrome is found more often in the female and would, therefore, be expected to excite predominantly female types of carcinoma. However, it is probable that, once initiated, the adrenocortical hyperfunction facilitates the growth and spread of the primary cancer. In animals, cortisone may facilitate the heterologous transplantation of tumors and the metastatic spread of primary tumors (39). In man, cortisone treatment had a deleterious effect on survival of patients with bronchogenic carcinoma when compared with no treatment and other forms of chemotherapy (39). Nabarro (26) mentions a case in which progression of the carcinoma slowed after total adrenalectomy.

Theoretically, a cancer might influence adrenal function mechanically via direct irritation of the adrenal cortex or anterior pituitary from metastases (23, 25, 38), or reflexly through vagal invasion (40, 41); or a cancer might secrete a polypeptide or protein with hormonal activity (1, 24, 25). The diversity of metastatic spread in cases so far reported argues against all mechanical theories. Furthermore, the spread is frequently duplicated by cancers so far not associated with Cushing's syndrome. The prevalence of a particular type of carcinoma (small cell undifferentiated bronchogenic carcinoma) in the series favors a humoral mechanism. Three types of hormone-simulating substances could produce the syndrome: a corticotropin-like polypeptide which directly stimulates the adrenal cortex; a substance which stimulates the hypothalamus; or a substance like corticotropin-releasing factor, which acts directly on the anterior pituitary. The corticotropin-releasing factor may be chemically similar to antidiuretic hormone (42); if so, the cases of oat cell carcinoma associated with inappropriate secretion of antidiuretic hormone (40, 43, 44) may be the result of humoral mechanisms related to those in cases of Cushing's syndrome. Instances of a patient (21) treated by pituitary irradiation with amelioration of symptoms and of another patient (30) who exhibited increased skin pigmentation suggesting increased corticotropin secretion favor a mechanism which acts through the pituitary. On the other hand, one writer (25) reports complete replacement of the pituitary by tumor.

Recent writers have suggested a general relation between cancer and adrenal hyperplasia: Werk and Sholiton (45) reported elevated cortical steroid production in cases of bronchogenic carcinoma; Parker and Sommers (46), in a study of post-mortem adrenals using adrenal cortical thickness as the criterion, found cortical hyperplasia associated more frequently with cancer than with other types of illness. Unpublished data from 142 autopsies performed at the West Haven Veterans Administration Hospital tend to support these writers. The patients of twenty-one cases, not including the case reported in this paper, had adrenal glands heavier than 18.3 gm., i.e., greater than one standard deviation from the mean weight of 14.3 gm. Combined adrenal weights in six cases exceeded two standard deviations from the mean, and five of these were cancer cases. In one case (the case being reported) the combined adrenal weight exceeded three standard deviations from the mean.

In another group of 50 autopsies, performed by one prosector at Grace-New Haven Hospital during the period 1958–1960, a similar mean combined adrenal weight and standard deviation were obtained (47). In this series, too, one case (combined adrenal weight 37 gm.) exceeded three standard deviations from the mean.

The patient was a 47-year-old white male (GNH #50-32-05, autopsy #A 13864) with small-cell undifferentiated bronchogenic carcinoma of the left main bronchus. Metastases involved the hilar and portal lymph nodes, pancreas, pericardium, esophagus, bone, prostate, and liver. The

patient survived six months from the presenting complaint of left shoulder pain. Although adrenocortical hyperfunction was not suspected, his course was complicated by an exacerbation of ulcer symptoms and "organic" psychoses attributed to cerebral metastases. No cerebral metastases were found at autopsy. It is likely that both the ulcer symptoms and psychoses were the manifestations of adrenocortical hyperfunction. (Psychosis was a prominent symptom in the case reported by Lewis (25).) Serum electrolytes were not measured in this patient. An electrocardiogram taken five months after diagnosis did not suggest hypokalemia. Fasting blood sugar was not elevated but blood pressure was moderately elevated, 180 systolic, 95 diastolic in mm. of mercury. The patient died following emergency surgery for massive hemorrhage from a duodenal ulcer. The adrenal morphology in this case is identical with that described in the present case report (figure 3).

It seems likely that the extraordinary hyperplasia found in most cases reviewed and in the two cases reported is distinct from the moderate hyperplasia described by Parker and Sommers (46) and present in some cancer and non-cancer cases in our own series. Moderate hyperplasia would be expected in chronic stressful illness. Rats treated with corticotropin show moderate hypertrophy, hyperplasia, and cortical lipid depletion, changes similar to those seen in stressed human adrenals (48). On the other hand, rats with functioning pituitary tumors (49) which produce corticotropin show a type of hypertrophy and hyperplasia morphologically similar to that noted in the patients with the cancer-Cushing syndrome. This suggests that the stimulus to adrenal hyperplasia present in the cancer-Cushing syndrome cases is of similar intensity, constancy, and specificity.

Summary

A case of Cushing's syndrome associated with small-cell undifferentiated bronchogenic carcinoma is reported. A second probable case is mentioned. The literature with respect to this interesting association and the more general combination of Cushing's syndrome and neoplasia is reviewed. It is concluded that in these cases the cancer is a specific stimulus to adrenocortical hyperfunction, that the adrenocortical hyperplasia is significantly greater than that usually found in Cushing's syndrome not associated with carcinoma, and that it constitutes the immediate threat to survival.

Sumario

Carcinoma Broncógeno con Síndrome de Cushing

Se presenta un caso del síndrome de Cushing asociado con carcinoma broncógeno no diferenciado de células pequeñas. Menciónase otro caso probable. Repásase la literatura relativa a esta interesante asociación y a la asociación más gene-

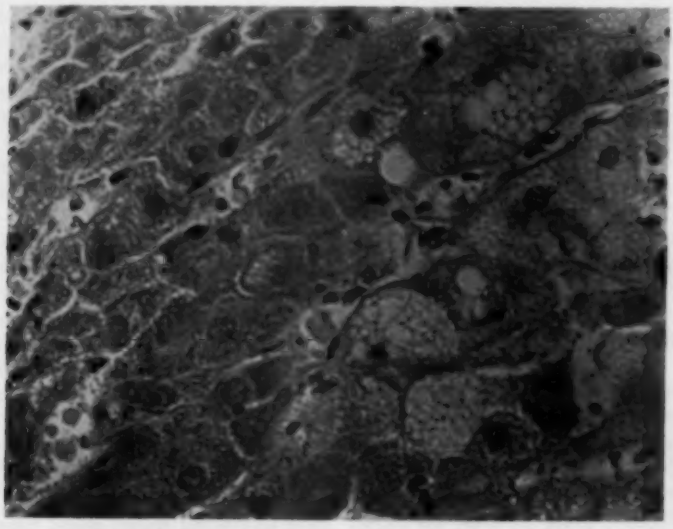

Fig. 3. Adrenal cortex—Case GNH (50-32-05) (×300)

ral del síndrome de Cushing y la neoplasia. Dedúcese que en estos casos el cáncer constituye un excitante específico de la hiperfunción córticosuprarrenal, que la hiperplasia córticosuprarrenal es significativamente mayor que la observada habitualmente en el síndrome de Cushing sin vinculación con carcinoma y que constituye una amenaza inmediata para la sobrevivencia.

Resume

Carcinome bronchogènique associé à un Syndrôme de Cushing

L'auteur rapporte un cas de syndrôme de Cushing associé à un carcinome bronchogènique à petites cellules indifférenciées. Il mentionne un second cas probable. Il donne une revue de la littérature concernant cette associçiation intéressante et l'associçiation plus générale du syndrôme de Cushing à un néoplasme. Il conclut que dans ces cas, le cancer est un stimuli spécifique de la fonction adrénocorticale, que l'hyperplasie adrénocorticale est nettement plus grande qu'on ne la trouve habituellement dans le syndrôme de Cushing lorsqu'il n'est pas associé à un carcimôme, et que ceci constitue une menace immédiate pour la survie.

Acknowledgments

The writer gratefully acknowledges the editorial comments of Drs. Patrick Mulrow and Thomas Amatruda, Jr., Medical Service; and Dr. Raymond Yesner, Laboratory Service, West Haven Veterans Administration Hospital. Special thanks are due to Dr. Jack Gallagher, formerly resident physician, Department of Pathology, Yale University School of Medicine, for providing additional autopsy data; and to Dr. Arnold Kisch, resident physician, Department of Surgery, Yale University School of Medicine, for assistance with translations.

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A SUMMARY OF THE THIRD CONFERENCE ON RESEARCH IN EMPHYSEMA[27, 28] AIR POLLUTION AND CHRONIC PULMONARY INSUFFICIENCY

(Concluded)

SESSION III. BIOCHEMICAL, PHYSIOLOGIC, IMMUNOLOGIC, AND GENETIC EFFECTS OF THE AIR POLLUTANTS ON LUNG TISSUES

Moderator: L. Hellerman

THE ACID MUCOPOLYSACCHARIDES OF CONNECTIVE TISSUES[29]

ALBERT DORFMAN

The formed elements of the connective tissues are embedded in an amorphous matrix, the ground substance. This complex colloidal solution contains both materials passing between cells and circulation substances peculiar to connective tissue. Included among the latter are the acid mucopolysaccharides. These high molecular weight polyelectrolytes are usually composed of alternating units of *N*-acetylated hexosamines, uronic acid, and sometimes sulfate. Their linear configuration, large molecular weight, and negative charge impart important physical properties, including high viscosity, water binding, and interaction with cations.

Eight different acid mucopolysaccharides have now been differentiated in mammalian tissues. Although these substances have structural similarities, they vary in chemical composition and biologic properties. Hyaluronic acid is composed of equimolecular amounts of *N*-acetylglucosamine and glucuronic acid, but contains no sulfate. Available evidence indicates that it is loosely bound to protein. Chondroitin sulfuric acids *A* and *C* contain equimolecular amounts of *N*-acetylgalactosamine, glucuronic acid, and sulfate. They differ only with respect to the position of the sulfate group which is substituted on the 4-position of the *N*-acetylgalactosamine in chondroitin sulfuric acid *A* and in the 6-position in chondroitin sulfuric acid *C*. Both substances appear to be bound to a protein core to form a macromolecule. Chondroitin sulfuric acid *B* differs from *A* and *C* in that D-glucuronic acid is replaced by L-iduronic acid, its *C*-5 epimer. This structural alteration profoundly affects the physiologic properties, as indicated by the fact that chondroitin sulfuric acid *B* in contrast to *A* and *C* has striking antithrombic properties.

The many biologic activities of heparin are well known, although its chemical structure has not as yet been fully elucidated. It contains 2 to 3 moles of sulfate per disaccharide unit composed of equimolecular amounts of *N*-acetylglucosamine and glucuronic acid. One mole of sulfate exists as a unique sulfonamide linkage. Heparin monosulfuric acid appears to be closely related to heparin, but differs with respect to a lower sulfate content and the presence of *N*-acetyl groups which are absent from heparin. It is present in large amounts in the urine and tissues of patients afflicted with the Hurler syndrome. Chondroitin is a low sulfate-containing substance related to chondroitin sulfuric acids *A* and *C* and has been regarded by some as a metabolic precursor of these compounds. Keratosulfate contains equimolecular amounts of *N*-acetylglucosamine, galactose, and sulfate; and these differ from the other acid mucopolysac-

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[27] From The Webb Institute for Medical Research in Tuberculosis and the Department of Medicine, University of Colorado Medical Center, Denver 20, Colorado, edited by Roger S. Mitchell, with the help of Giles F. Filley and Morgan Berthrong.

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[29] From LaRabida University of Chicago Institute and Department of Pediatrics, University of Chicago School of Medicine, Chicago, Illinois.

charides by the presence of a neutral sugar instead of a uronic acid. It has recently received considerable attention because of the apparent replacement, with advancing age, of the chondroitin sulfuric acids by this substance in cartilage.

The diversity of acid mucopolysaccharides has suggested that physiologic and pathologic alterations of tissues may coincide with both qualitative and quantitative changes in acid mucopolysaccharides. To examine this possibility, a method has been devised for the determination of small amounts of polysaccharides in tissues. This depends on the differential solubility in salt solutions of complexes of the polysaccharides and the cationic detergent, cetyl pyridinium chloride. This method has been applied to the study of polysaccharides in skin, aorta, and lung. Surprisingly large amounts of heparin were found in rat skin. The concentration of heparin decreases with age. Alloxan diabetes results in a marked decrease in the concentration of both hyaluronic acid and chondroitin sulfuric acid and little change in the heparin content.

These results suggest that intensive study of the composition of connective tissue ground substance by appropriate methods may reveal changes significant to the understanding of physiologic and pathologic mechanisms.

BIOCHEMICAL STUDIES ON DIFFERENT ELASTINS[20]

MARTIN J. FITZPATRICK AND
VERNE D. HOSPELHORN

There are two principal difficulties involved in any biochemical study of elastic fibers. These are, first, the constant and intimate association of elastin with other fibrous connective tissue elements such as collagen and reticulin and, second, the great resistance of elastin to isolation from this connective tissue by any but the most drastic chemical techniques. In our laboratory we have been studying methods of recovering elastic fibers from different connective tissues by a number of approaches. Data are presented to show that significant differences in the final product may occur.

One technique of isolation of elastin is a modification of the classical digestion technique of Lowery and associates based on alkaline degradation of other connective tissue components (1). Normal lung is ground and chopped, then washed with water and hypertonic saline, and defatted with butanol and acetone. This yields a fraction called crude connective tissue, which is ground into a fine powder. It consists principally of collagen, elastin, and reticulin. This crude connective tissue is then subjected to alkaline digestion to yield a residue, base elastin. When dried, this constitutes 12 per cent of lung connective tissue and 35 per cent of connective tissue from the aorta.

An alternate procedure, based on proteolytic enzymes which do not attack elastic fibers in the normal state, has been utilized in this laboratory. The crude connective tissue is subjected to alternate digestions with crystalline trypsin and collagenase until no further protein is liberated. The product is dried and yields a fraction called enzyme elastin. This constitutes 17 per cent of lung connective tissue by weight and 40 per cent of connective tissue from the aorta.

The dried elastins are hydrolyzed in 6 N hydrochloric acid at 100°C. for sixteen hours, and appropriate quantities of the hydrolysates are introduced into a Spinco amino acid analyzer. A complete amino acid "profile" of the elastins is thus obtained. Nitrogen content is determined by the classical Kjeldahl method.

On comparing amino acid composition of base and enzyme elastin from dog lungs, it is noted that there is a reduction in serine, threonine, methionine, and arginine in the base elastin. In addition, there is also a significant loss of aspartic acid, glutamic acid, and histidine. Other studies in this laboratory have demonstrated that there is a striking difference in the rate of attack by elastase on base and enzyme elastin. The latter is more resistant to elastolysis by elastase and also by papain.

Base elastins recovered from the aorta and from the lung in the dog were compared and found to be similar. On comparing base elastins from human and dog lung, it was found that there were significant differences in glycine, alanine, and aspartic acid content. Dog elastin has a higher content of glycine and alanine, but a lower content of aspartic acid, than does human elastin. Also there was a significant difference between man and dog in the amount of nitrogen recovered as amino acid.

Application of the enzymatic procedure to the connective tissue of the human lung does not yield an elastic tissue preparation. The residue obtained contains, in addition to elastic fibers, fibers which stain like collagen, have an amino acid composition similar to collagen, but are resistant to attack by collagenase.

In summary, we have presented data which indicate that: conventional alkaline digestion of connective tissue yields an elastin with several significant differences in amino acid composition; elastins from the same species do not differ greatly from one organ system to another in amino

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acid composition; and human lung connective tissue contains a fibrous structure which appears to be quite similar to collagen but resists digestion by collagenase.

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HEXOSAMINE CONTENT OF AGED AND CHRONICALLY DISEASED LUNG[21]

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The occurrence of emphysema in aged and chronically diseased lung suggests that alteration in the connective tissue supporting structures of this organ may be an important factor in the pathogenesis of the disease process. A decreased collagen-to-elastin ratio in aged lung has been reported by others, but a significant difference in collagen content between the aged and the emphysematous lung was not noted. Alterations in lung hydroxyproline content which correlate with age have also been described. Observations have not been made, however, on the hexosamine content of connective tissue in the aged or diseased lung. In studies on cartilage and nucleus polyposus, it has been demonstrated that hexosamine content varies significantly with age. It was the purpose of this study to determine hexosamine content and its primary components, galactosamine and glucosamine, in different species at various ages and in diseased human lung.

Method: Lung parenchyma was obtained from healthy rats, dogs, guinea pigs, and rabbits of various ages. Human pulmonary parenchymal tissue was obtained at necropsy. Histologic and biochemical observations were made on each specimen. The human material was classified microscopically into four groups: normal; emphysema and fibrosis; acute changes of edema, congestion, and pneumonia; mixed acute and chronic changes.

The tissue for biochemical analysis was acetone dried, hydrolyzed in dilute acid, filtered, and placed on short Dowex #50 ion exchange resin columns. Hexosamine was eluted off with strong acid and measured colorimetrically. A portion of the same hydrolysate was placed on long Dowex #50 resin columns, and galactosamine and glucosamine were separated by a time acid gradient elution into serial 2 ml. fractions.

Results: Average values for hexosamine content of healthy adult mammalian lung tissues were 0.771 mg. per 100 mg. dry weight for rats, 0.704 for dogs, 0.583 for rabbits, 0.642 for guinea pigs, and 0.652 for man. The galactosamine-glucosamine ratio for the normal human lung was 0.223.

Hexosamine content at various ages was determined on rabbit lung tissue. The mean values were 0.503 mg. per 100 mg. dry weight at birth, 0.520 at two weeks, 0.557 at two months, 0.550 at four months, 0.540 at eight months, 0.566 at eighteen months, and 0.583 at two years. Galactosamine-glucosamine ratios on the same specimens averaged 0.19 at two months and did not vary more than plus or minus 0.040 at the other ages.

Pulmonary tissues from 51 patients with pathologic evidence of lung disease were studied. Specimens from 15 persons with both clinical and pathologic evidence of emphysema showed a mean hexosamine content of 0.571 mg. per 100 mg. dry weight with an average galactosamine-glucosamine ratio of 0.249. Eight specimens, however, showed a rise in the galactosamine-glucosamine ratio of more than one standard deviation above the normal value with levels as high as 0.340. Observations on specimens from 18 subjects with acute histologic changes showed a mean hexosamine content of 0.782 mg. per 100 mg. dry weight, and the mean galactosamine-glucosamine ratio was 0.17. In 11 of the specimens the ratio was more than one standard deviation below the mean for normal lung tissue. In the remainder of the specimens showing a mixture of acute and chronic inflammatory changes and emphysema, hexosamine and its components did not differ quantitatively from the normal range.

Discussion and conclusions: From this study it is apparent that normal lung tissue contains relatively small amounts of hexosamine. There was little variation in the hexosamine content of normal animal and human lung tissue. Moreover, the components, galactosamine and glucosamine, did not differ quantitatively with a species or age change. It appears likely, therefore, that age-determined alteration in hexosamine or galactosamine-glucosamine content of human lung does not occur.

The trend toward higher ratios of galactosamine to glucosamine in emphysematous lung suggests the possibility of significant alteration. However, because the ratio fell with a rise in hexosamine content in the lung tissue showing acute inflammation and congestion, the possibility should be considered that the blood contained in the specimens analyzed may have been responsible for the changes observed.

The lung hexosamine alterations observed in

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this study may have contributed to the associated histologically evident disease, but they could also have been an incidental finding due to change in pulmonary blood content. Further studies of isolated lung structure such as tracheobronchial cartilage and an evaluation of pulmonary connective tissue by other means may help clarify this problem.

STUDIES ON THE EFFECT OF SULFUR DIOXIDE ON CILIARY ACTIVITY IN RABBIT TRACHEA IN VIVO AND IN VITRO AND ON THE RESORPTIONAL CAPACITY OF THE NASAL CAVITY[*]

TORE DALHAMN

For the first experiments live rats were used.

With lengthwise incised trachea, the animals were placed in a moist chamber. The rate of ciliary beat was registered with a high-speed motion picture camera (200 pictures per second). When the film was projected the seemingly slower rate of beat could be calculated and then computed to the true value.

The speed of mucus flow in the trachea was measured by the simple method of timing the passage of cells, et cetera, in the mucus between two points on a scale inserted into the ocular of the microscope. The action of some respiratory irritant gases on the trachea was investigated by exposing rats to about 10 p.p.m. sulfur dioxide daily for one to two months and at the end of that time measuring the speed of ciliary movement and of mucous transportation.

Concerning the effects of the functions in question, they may be summarized by saying that definite lasting alteration of ciliary activity was not seen, but the flow of mucus ceased in some rats and was greatly decelerated in others.

Morphologic investigations from trachea of rats exposed to sulfur dioxide (10 p.p.m., 6 weeks) showed that the cell surface toward the lumen of the trachea was extremely uneven and showed deep crypts. The mucosa and submucosa were fragmented by an edematous vascularized layer.

A functional and morphologic mucosal reaction was thus evoked by continuous exposure to low concentrations of sulfur dioxide. Single exposure showed that 10 p.p.m. of sulfur dioxide caused cessation of ciliary activity after about five minutes; ammonia and formaldehyde in a concentration of 10 p.p.m. stopped the activity in about one minute.

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To make the registration of ciliary activity more physiologic, some alterations were carried out. These alterations made it possible for the animal to breathe through the normal respiratory passages. The inhaled air was then moistened and warmed in the ordinary way and, furthermore, the filtration and resorption action of the nose was not dispensed with but also functioned normally. Finally, the method can be used for larger animals than rats; for instance, rabbits and cats.

The mean frequency of ciliary movement in 31 living rabbits was 1,099 beats per minute. The interanimal deviation was 172 beats per minute and the error of method, 40 beats per minute.

The next step was to allow rabbits to inhale various concentrations of sulfur dioxide, using the modified method and simultaneously recording ciliary activity.

It was found that the concentration of sulfur dioxide in the inhaled air had to reach about 200 to 250 p.p.m. before ciliary beating ceased. For the experiments proper, therefore, 100, 200, and 300 p.p.m. of sulfur dioxide were selected. Each concentration was tested on a series of 10 rabbits. In no rabbit did 100 p.p.m. of sulfur dioxide cause cessation of ciliary movement. When the concentration of the gas was raised to about 200 p.p.m., the ciliary beating ceased within forty-five minutes in 6 of 10 rabbits. A concentration of 300 p.p.m. of sulfur dioxide caused tracheal ciliary movement to cease in 9 of 10 rabbits within forty-five minutes.

Here we have an apparent contradiction of the observations when sulfur dioxide was blown directly over the trachea of rats. For this difference, at least two explanations seemed possible. First, the rabbit mucosa might be appreciably less sensitive for sulfur dioxide than rat trachea. The second and more likely explanation was that a major proportion of the gas was removed in the rabbit nostrils, leaving only a small amount to enter the trachea.

To elucidate this question, 12 isolated rabbit tracheas were exposed *in vitro* to a single exposure of sulfur dioxide under the same conditions as the rats. If the important sources of error in the method are taken into account the results may be said to tally on the whole with the findings in rats, and no essential difference was observed which could explain the wide discrepancy in the studies on live rabbits.

The explanation therefore was to be sought in the resorption function of the rabbits' nostrils. This function was studied with a method which, although only approximate, gives some idea of the power of resorption.

The gas-air mixture was continuously sucked

through the nose or mouth for forty-five minutes, and analyses were made on samples collected from an intratracheal cannula fifteen, thirty, and forty-five minutes after the exposure began. Each sulfur dioxide concentration was tested on 10 rabbits.

When the rabbits were exposed to about 100 p.p.m. of sulfur dioxide, only 4 to 6 p.p.m. remained after passage through the nose. When the inhaled concentration was raised to about 200 p.p.m., the amount of sulfur dioxide recovered from the trachea was still low—6 to 8 p.p.m. Increase to 300 p.p.m., however, brought about an appreciable rise in the concentration that had passed the nostrils; this was now 25 to 30 p.p.m.

As I have pointed out, some of the methods of exposure to gas are clearly imperfect and, therefore, conclusions based on the reported concentrations and times should not be pressed too far. In my opinion, some of the more interesting results were the following. It has been possible to register ciliary activity in the trachea in animals breathing through the normal respiratory pathways and to calculate statistically the error of method and the interanimal deviation. With this method it was found that the concentrations of sulfur dioxide in rabbits which must be inhaled before effect on ciliary beating in the trachea becomes discernible are many times as high as those that occur in smog, for example. This does not preclude the possibility that much lower concentrations would produce an effect on the cilia in the nasal cavities. Between rabbits and rats no essential difference in the mode of reaction of the tracheal cilia to sulfur dioxide was found. By simple experiments it was demonstrated that the mechanism of resorption in rabbit nostrils is highly efficient.

To judge from the results in these two series of experiments—when sulfur dioxide was blown directly over the tracheal mucosa and when the efficiency of nasal resorption was tested—it could be anticipated that in inhalation experiments it would be necessary to expose the animals to relatively high concentrations of sulfur dioxide in order to produce an effect on the ciliary beating in the trachea. This assumption proved to be correct, and thus there was fairly good agreement between the results in all three experimental series.

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METABOLIC FUNCTION IN RESPIRATORY MUCOSA AS STUDIED IN VIVO AND BY ORGAN CULTURE[a]

T. TIMOTHY CROCKER

The finding of principal interest for this conference was that there appears to be a rapid passage of $S^{35}O_4$-labeled material through the epithelial cells—other than goblet cells—of the jejunum, tracheal glands, bronchi, and alveoli of the rat. This presumed secretory activity was identified during experiments designed to compare metabolic functions of rat respiratory tissues *in vivo* and in cultures of organs.

Data pertinent to secretory behavior were obtained in living rats receiving inorganic $Na_2S^{35}O_4$ intraperitoneally at intervals of one-half, two, four, eight, twenty-four, and forty-eight hours before killing, followed by fixation of tissues in acetic acid:ethanol (1:4), then neutral formalin:ethanol (1:10) at room temperature. Paraffin sections were hydrated, soaked in unlabeled 2 per cent Na_2SO_4 for two hours prior to filming for autoradiography by the stripping film method of Pelc. Grain counts over epithelia, connective tissues, cartilage, and luminal contents of glands or respiratory airways and alveoli were performed by Miss Beryl Ingram, Mr. Thomas McClure, and the writer to estimate the rate of movement of label into, and out of, these structures.

Three different rates were observed. First, acine but not ducts of tracheal glands reached peak concentrations at two hours and were nearly empty at eight hours. Second, epithelial cells, other than goblet cells, labeled maximally at four hours, remained at this level at eight hours and cleared essentially completely at twenty-four hours. As

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label declined in epithelial cells, it was observed to increase in secretions overlying the cells. Alveolar walls and a thin layer of alveolar secretion seemed to follow this pattern. Goblet cells acquired label on this pattern but many remained lightly labeled at twenty-four and forty-eight hours. Third, tracheal cartilage and connective tissue surrounding bronchi acquired maximal label by eight hours and retained most of this label at forty-eight hours.

Organ cultures of rat trachea and lung by the Trowell method (1) confirmed these data at oxygen concentrations of 20 and 95 per cent but did not demonstrate labeling at 0.2 per cent.

Interpretations from these preliminary data are not yet final. At present we presume that the labeled SO_4 was incorporated into mucins and mucopolysaccharides which differ in rates of formation or turnover. The cartilage and connective tissue data are compatible with data reviewed by Dziewiatkowski (2). The course of goblet cell labeling corresponds essentially with observations of Jennings and Florey (3) for the intestine. Unexpected observations, requiring further analysis, were presented by the presence of S^{35} among non-goblet epithelial cells of the gut, trachea, bronchus, and alveolus. While the rapid appearance and disappearance of label from these cells is associated with appearance of label in the adjacent secretions, it remains to be demonstrated that a sulfated mucin or mucopolysaccharide is actually formed with this speed at these sites.

Most epithelial mucins so far studied do not contain ester sulfate and should not therefore be labeled by recently administered inorganic sulfate. Even if S^{35} is attached artificially but irreversibly to a normally nonsulfated secretion, however, labeling might serve as a useful indirect indication that rapidly formed secretions exist. Secretions of the respiratory tract have not yet been studied in many species, and the autoradiographic method may serve as a useful guide to both chemical composition and rate of formation of secretions in future studies.

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THE BIOCHEMICAL AND PHYSICAL PROPERTIES OF EPITHELIAL MUCUS[24]

R. A. Gibbons

Chemical properties: The chief constituent of epithelial mucus is a blood group substance like mucosubstance of fucomucan. This contains fucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, neuraminic acid, and some 12 amino acids (1); their chemical structure is largely unknown; the molecular weight is high ($>4 \times 10^6$) (2). The polysaccharide moiety, which constitutes the greater part (75–90 per cent) of the molecule, varies in composition and structure with species and shows intraspecies differences also. These fucomucans are the blood group substances; their serologic properties, and therefore chemical structure, are controlled by the appropriate genes; there is some information on the chemical structure of the serologically reactive groups which involves only the neutral sugars and the two hexosamines (3). The amino acid part of the molecule is highly characteristic and surprisingly similar in the few species studied. The aromatic and sulfur-containing amino acids are low or absent; serine, threonine, and proline are high. With care, the fucomucan may be isolated as a cohesive elastic gel similar physically to the native mucus. Procedures are available for obtaining the fucomucan as a soluble preparation, but these usually involve the removal of much of the neuraminic acid, yielding a neutral mucopolysaccharide of relatively low molecular weight (about 2×10^6). Material of this type has been carefully studied, but it does not usually exist as such in the native mucus.

Protein, the major part of which is immunologically identical with serum protein, is also present in mucus, although in variable amounts. The gamma globulin may be specifically immune in infections involving the mucous epithelium (4). The ions and other low-molecular weight substances are not usually remarkable; the mucus is usually about isotonic with serum and of a pH close to neutral. Na^+ and Cl^- ions predominate.

Other mucosubstances are also present in some secretions; they are usually minor components. Acidic mucopolysaccharides, of the type described by Dorfman, are reported in gastrointestinal mucus but probably do not occur elsewhere. Sialomucan is present in saliva and mucoprotein in most mucous secretions; the former is made up of polypeptide, *N*-acetylgalactosamine, and neuraminic

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acid, and the latter consists mainly of protein with a relatively small polysaccharide portion consisting usually of the same sugars as those found in fucomucan, with mannose in addition. Furthermore, many other substances, such as enzymes, having usually specific functions at the site of secretion, are found in the native mucus.

Physical properties: It has been shown that the fucomucan of epithelial mucus is a long, flexible, threadlike molecule of "random coil" form (2). The physical chemistry of this type of molecule has been carefully investigated by the polymer physicists (5), whose work shows that the physical properties of native mucus may be interpreted in terms of the thermodynamic properties of such molecules. Thus, solutions of such molecules will separate into two phases, and the gel phase will form a random network. Such a network would be cohesive and elastic, would show the Weissenberg effect, and would be able to be drawn out into long elastic threads. All of these properties are rather strikingly characteristic of epithelial mucus and of the purified fucomucan which can be separated from it. Differences in consistency of the mucus are due in the main to differences in degree of coiling. In the very thick mucus, the molecules are tightly coiled, whereas the thinner secretions contain relatively open molecules; quite small changes in the degree of coiling give rise to marked changes in the consistency of the native secretion. These differences are presumably reflected in the chemical structure of the fucomucan affecting the rigidity of the thread and/or the interaction parameters which determine the degree of coiling. However, chemical investigation shows that fucomucan isolated from secretions of differing consistency does not differ greatly in composition, the only obvious difference being in the neuraminic acid content which, rather surprisingly, is higher in the fucomucan isolated from the thicker mucus. Other workers have noted that higher neuraminic acid values in crude mucus are associated with a thicker secretion, and it has been reported that sputum from patients with bronchial infections is higher in neuraminic acid than sputum from normal persons (6). This is probably a reflection of the difference in consistency also. Enzymes which cause a rapid change in the consistency of mucus are well known. Many of these are proteolytic (ficin, pepsin); others are unresolved mixtures of carbohydrases, e.g., *trichomonas foetus* lysates, *clostridium Welchii* filtrates. The neuraminidase of *vibrio cholerae* filtrates and the receptor-destroying enzyme of influenza virus bring about the hydrolysis of the neuraminic acid residues.

Injury of mucous epithelium by mechanical means, by infection, or by the action of chemicals, including probably some of those responsible for air pollution, brings about the thickening of the mucus secreted. The consistency of the cervical mucus is dependent on the female sex hormones; other mucous secretions do not change with the sexual cycle in so marked a manner. Nevertheless no controlled study of the effects of hormones on mucus has yet been made. The processes whereby these agencies bring about chemical changes in the mucus are quite obscure. Indeed, it may be seen from the foregoing that we have only fragmentary knowledge of the chemical differences underlying the differences in consistency of the mucus, although some conclusions may be drawn as to the fundamental physics of the problem. To clarify the position, detailed structural chemical information is what is ultimately required, but this appears to be a formidable problem in carbohydrate chemistry. At present, it seems most profitable to learn what one can of the large-scale molecular architecture of fucomucans, making use of all of the techniques—immunologic, enzymologic, physical, and chemical—which are currently available.

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IMMUNOLOGIC CHANGE ASSOCIATED WITH THE DEVELOPMENT OF THE CHRONIC PULMONARY LESION[m]

Lester D. Scheel

In the development of knowledge concerning the mechanism by which chronic lesions of the

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lung develop in various disease states, the observation of an associated immunologic change has often been made. The hypothesis which is being made here is not an entirely new idea, but is rather an extension of an old idea which enjoyed its greatest acceptance during and after Jenner's demonstration that cowpox would protect humans against the act of infection due to smallpox.

This hypothesis suggests "that the antigenic causes for the development of immunologic changes in the cells surrounding the injured tissue determine the extent of the injury and may act as a stimulus to healing by fibrous tissue replacement." Embodied in this hypothesis is the suggestion that the antigenic properties of a substance can be considered as a toxic property of the substance.

It is an accepted fact that biologic substances such as foreign proteins are antigenic, but it is not so widely accepted that nonbiologic substances which react with the host tissues produce antigenic substances. To illustrate this point, let me review briefly certain pertinent observations regarding what is now known about the development of the silica lesion.

In December, 1953, the Biochemistry Department of the Trudeau Foundation published a paper (1) concerning the toxicity of silica solutions. In this paper the writers defined silica solutions as those which contained silicic acid in only monomolecular dispersion. During this study it was shown that colloidal silicic acid could not form in solution unless the concentration of silica exceeded 168 mg. per liter. This observation was confirmed by the English workers in 1956 (2). The Trudeau Foundation work also showed that no reaction of monomeric silicic acid could be demonstrated with protein *in vitro* or *in vivo*. Their conclusion at the end of this paper was: "Because of the lack of demonstrable toxicity obtained with soluble silica, it is necessary to find another explanation for the toxicity of silica in tissues as manifested in the disease silicosis."

In the January issue of *AMA Archives of Industrial Health*, 1954 (3), an alternative basis for the toxicity of quartz dust was presented, namely, that the dust particle adsorbed protein on its surface and that in some manner this film of protein served as a stimulus for the proliferation of fibrous tissue. Since the adsorbed protein could be shown to possess an altered antigenicity, and since the injection of quartz particles into rabbits was shown to produce changes in the serum which caused agglutination of protein-coated quartz particles, the writers suggested: "The alteration of protein structure in the adsorbed film is clearly shown and it is entirely possible that the toxic action of the quartz particle may be the result of a foreign protein reaction in tissues."

These observations have been repeatedly confirmed and extended by several laboratories. This immunochemical change was characterized in man by Antweiler and Hirsch in Germany in 1957 (4). It was shown by using protein-coated silica particles that the agglutination is much stronger than with uncoated silica particles. Using nonsilica particles, the agglutination was negative whether the particles were protein-coated or not. Therefore, a specific change was established in the surface of the silica particle after protein adsorption. Antweiler and Hirsch extended our knowledge of this phenomenon to man by performing their agglutination test in a well-controlled experiment, using human serum.

In Italy, Dr. Vigliani and his group of co-workers at the Industrial Institute in Milan (5) have added other data to the evidence that antigen-antibody reactions are apparent in the silicotic nodule. They have shown that fluorescent-labeled antibodies from the silicotic animal will react with fibrotic silica lesions *in vitro*, thus establishing that antigen molecules are present in this tissue. Such antibodies do not react with amyloid lesions, again establishing a *silica* specificity.

In summary, we may rationalize the results for the silicotic lesion in this way. On the basis of biochemical and immunochemical results to date, the suggested stimulus for the proliferation of fibrotic tissue observed in the development of the silicotic lesion may be the result of immunochemical changes in cells around the lesion.

This suggestion is even more attractive when consideration is given to the adjuvant or synergistic effect of tubercle bacilli and silica acting together in the same tissue to produce fibrous lesions.

In the previous discussion I have cited a few of the pieces of evidence indicating the interaction of a solid surface and tissue components and the immunologic change which accompanies the development of a fibrous lesion. I wish now to cite the evidence that ozone reacts with proteins to form an altered protein structure which also ultimately results as fibrous lesions in the lung.

In July, 1958 (6), the Occupational Health Field Headquarters in Cincinnati published a paper in which the action of ozone on proteins was studied. The writers showed that ozone altered the protein structure and that animals exposed to ozone produced antibodies which gave strong precipitin reactions with ozonized protein, weak precipitin reactions with heat-denatured protein, and no reaction with native protein as determined by the precipitin tests. After repeated insults with ozone,

the exposed lung tissue showed localized fibrous lesions scattered about the lungs, usually located at the bronchiolar-alveolar duct junction.

During examination of the sequential pathologic changes seen in the early stages during the development of the two lesions, similarities can be observed. Let me illustrate this point.

The sequential pathologic changes during the development of the quartz lesion have been described by Schepers (7) as follows: (*1*) infiltration of the dust deposit and the surrounding tissue by the leukocytes; (*2*) migration of histiocytes into the area; (*3*) death and disintegration of the histiocytes; (*4*) deposition of fibrocytes in the dust-laden area; and (*5*) fibrous tissue growth around the dust deposit. This pattern of events has been confirmed by two other laboratories (2, 8).

In the case of ozone—a very reactive chemical—repeated insults to the lung tissue lead to the following pattern of pathologic change: (*1*) cuffing of leukocytes at the bronchiolar-alveolar duct junction; (*2*) macrophage, epithelial, and fibrotic infiltration of alveolar septa; (*3*) the deposition of fibrous tissue in the injured area; and (*4*) parenchymal lesions distributed throughout the lung.

In view of the similar changes found in the development of these two lesions resulting from completely different forms of stimuli: one, physical adsorption and structural alteration of protein; and, another, chemical alteration of protein structure, and other observations of related changes reported by Gross (9), it is suggested that the immunologic change induced by the altered protein structure may be a necessary and normal part of the fibrous healing process of a chronic lesion.

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DO AIR POLLUTANTS ACT AS MUTAGENS?*

Hermann J. Muller

An increasing number of toxic substances that may occur as pollutants have been found to produce their chief damage by way of a mutagenic action, that is, by injuring the genetic material of the cells they enter. Some substances produce this kind of effect indirectly by giving rise to chemical reactions wherein a mutagenic substance is produced. Among the more directly mutagenic substances are those with peroxide, epoxide, chlorethyl, ethylene-imino, and mexyloxy groups. In general, these are especially active when two such groups occur in the same molecule, possibly because of the greater likelihood thus afforded of both strands of the Watson-Crick deoxyribonucleic acid doublet (chromosome) being affected simultaneously at nearby points.

Thus far, all chemical mutagens intensively studied have been found to produce the same general complex of clinical effects on tissues into whose cells they penetrate, as do ionizing radiation and ultraviolet; that is, they are radiomimetic. Thus, like radiation, they produce irritation, inflammation, necrotic and precancerous changes, particularly in the more rapidly dividing cells of the parts penetrated, and they finally give rise there to malignant neoplasms. Again like radiation, however, they tend to be more injurious to malignant than to normal tissue, in correspondence with the former's faster proliferation, and they thereby act not only carcinogenically but also carcinolytically.

The carcinogenic effect is probably an expression of the production in individual genes of individual somatic cells penetrated and of mutations that reduce the cell's susceptibility to inhibition of its proliferation. In cases in which the agent reaches the germ cells, the mutations induced by it are manifested mainly as inheritable impair-

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ments, usually slight but of the most diverse kinds, that afflict the descendants through an indefinitely long succession of generations. On the other hand, the irritating and destructive effects of mutagens (including their carcinolytic action) on organisms exposed to them are expressions of the genetic impairment and death of scattered somatic cells that have experienced chromosome loss of nuclear entanglement, in the course of mitoses undergone after one or more of their chromosomes had been broken by the mutagen. Recent studies indicate that the generalized long-term rise in morbidity and mortality, amounting to a decrease in longevity, of persons exposed to penetrating mutagenic agents, are also expressions of scattered cell losses caused by chromosome breaks.

Many mutagenic substances are destroyed or rendered innocuous before attaining circulation in the body, as, for example, when a peroxide molecule is attacked by catalase; but sometimes such defenses are incomplete or may be used up by near-saturation doses. Especially in the portals of entry, such as the lungs in the case of air pollutants, the concentrations would be more likely to be high enough to succeed in reaching the cell nuclei and there exert their mutagenic influence. In such cases the chief visible signs of their action would be a shorter term of irritation and edema of the organ of entry, a longer term of scarification and reduction in its functional capacity, vigor, and resistance to challenges, and ultimately, in some cases, carcinogenesis.

SESSION IV. SURVEY METHODS FOR THE DETECTION OF CHRONIC PULMONARY DISEASE

Moderator: G. Filley

AIR POLLUTION AND CHRONIC RESPIRATORY DISEASE[#]

STANLEY N. ROKAW

This preliminary report covers the first year of a study of the effects of environmental factors on pulmonary performance, including air pollution. The subjects have been male inpatients with severe, disabling, respiratory disease and persons with no known respiratory impairment, selected from hospital personnel. Functions tested among the patients included full vital capacity (VC), timed vital capacity (TVC), air flow velocity during cough and forced expiration, rebreathing and end-tidal mean capillary oxygen pressure (PCO_2), monthly functional residual capacity (FRC), daily sputum volume, and subjective well-being. For the "normal" group only full vital capacity and timed vital capacity were recorded. Environmental variables monitored include temperature, humidity, oxides of nitrogen, carbon monoxide, ozone, total oxidant, oxidant precursors, and particulate matter. A program for statistical analysis has been developed employing the IBM 709 computer at the Western Data Processing Center, University of California, Los Angeles.

Approximately 25 patients and 12 "normal" subjects have been serially tested during the past year. The number of test experiences for many of the patients has reached the range of 130 to 160 trials. In most of the normal group, 70 to 90 test experiences have been recorded. Gaps in test data have been occasioned by illness, passes or discharge from the hospital, vacations and, rarely, by equipment breakdown.

Preliminary review of our test data raises several interesting points in addition to the study of dependence on environmental factors:

1. Wide daily variability within a specific pulmonary function test. The day-to-day changes between recorded values of vital capacity and timed vital capacity were greater than is usually reported for these tests. This was most marked in the patient group, was present to a lesser extent in our older normal subjects, and was almost within the expected range in the young normal subjects.
2. Absence of correlation of changes between the several tests of pulmonary function.
3. Absence of specific pattern of change foreboding the death of patients. For many of the patients, however, there has been a suggestion through the year of rising mean capillary oxygen pressure and gradual decline of vital capacity values.

We tried to determine whether the wide variability of test results was indeed dependent on the several environmental factors being monitored. Tables of correlation coefficients (table 1), both single and multiple, against each of the vital capacity and the three-second timed vital capacity measurements have been prepared for many of the subjects. Environmental variables plotted have included temperature, carbon monoxide, and oxidants; these were measured at the time of testing, for several time periods preceding tests, and during various combinations of these three pe-

[#] From the Rancho Los Amigos Hospital, Downey, California.

TABLE 1

AIR POLLUTION STUDY

CORRELATION COEFFICIENTS, SINGLE AND MULTIPLE

I. Vital Capacity vs. Environmental Variables

Case Number	Temperature (Test Time)	CO* 72-hour Total	Temperature + CO(−72)	Temperature + CO + Oxidant (72)	Temperature + CO (24, 48, 72 hour)	Temperature + CO + Oxidant (Test)	CO (Test Time)	(Oxidant (Test Time)
51	0.314	−0.136	0.337	0.488	0.355	0.452	0.271	0.409
52	−0.185	0.332	0.354	0.371	0.427	0.284	0.404	−0.024
53	0.354	−0.191	−0.382	0.533	0.393	0.515	0.197	0.510
54	−0.194	0.009	0.199	0.544	0.321	0.222	−0.161	−0.206
55	0.233	−0.230	0.354	0.378	0.616	0.487	0.053	0.423
56	−0.088	−0.419	0.461	0.466	0.462	0.272	−0.003	0.118
57	−0.050	−0.032	0.071	0.163	0.134	0.125	0.123	−0.017
58	−0.132	−0.035		0.144	0.301	0.226	−0.176	−0.067
59	0.410	−0.109	0.420	0.575	0.421	0.451	0.046	0.208
60	0.159	−0.145	0.198	0.317	0.404	0.220	−0.198	0.060
61	0.281	−0.262	0.362	0.436	0.396	0.327	0.047	0.214
2	−0.380	−0.155	0.388	0.403	0.432	0.476	−0.084	−0.392
6	0.028	−0.357	0.377	0.467	0.442	0.231	−0.199	−0.125
9	−0.221	−0.638	0.639	0.653	0.641	0.558	−0.547	
11		−0.251					0.107	0.218
13	−0.293	−0.114	0.318	0.375	0.439	0.359	0.171	−0.112
20	0.146	−0.257	0.377	0.524	0.383	0.333	0.060	0.301

II. Timed Vital Capacity vs. Environmental Variables

51	0.373	−0.073	0.378	0.390	0.434	0.403	0.119	0.328
52	−0.237	0.367	0.405	0.410	0.479	0.442	0.399	−0.056
53	0.247	−0.233	0.318	0.469	0.455	0.402	0.027	0.383
54	−0.051	0.362	0.381	0.670	0.593	0.191	0.482	0.105
55	0.416	−0.212	0.498	0.580	0.640	0.434	0.050	0.272
56	0.006	−0.457	0.469	0.487	0.470	0.263	0.010	0.196
57	−0.038	−0.023	0.052	0.103	0.146	0.108	0.029	0.042
58	0.003	0.105		0.422	0.348	0.225	0.224	0.070
59	0.410	−0.109	0.420	0.575	0.421	0.451	0.046	0.208
60	0.366	−0.209	0.394	0.459	0.462	0.381	0.068	0.224
61	0.174	−0.217	0.263	0.304	0.300	0.216	0.050	0.160
2	−0.343	−0.444	0.457	0.465	0.499	0.397	−0.328	−0.353
6	−0.133	−0.552	0.553	0.612	0.607	0.418	−0.106	−0.306
9	−0.172	−0.739	0.749	0.765	0.779	0.626	−0.599	−0.301
11		−0.138					0.137	0.233
13	−0.163	−0.285	0.333	0.375	0.463	0.265	−0.064	−0.253
20	−0.037	−0.193	0.199	0.505	0.230	0.331	0.081	0.184

* CO—carbon monoxide.

riods. No single environmental variable (save for carbon monoxide in *Case 9*) correlated well with the observed variations in performance of pulmonary function tests. There are, in several cases, "interesting" correlation numbers in which multiple factor effects have been calculated, i.e., temperature plus seventy-two-hour carbon monoxide. But the combination of factors which seems to produce such an "interesting" (as distinct from "significant") number differs for the several persons who have been studied. Perhaps one of the measurements of environmental carbon monoxide is the most strongly related to pulmonary variability.

At this stage in our data analysis, no better or firmer point of interdependence has been found (even for other functions measured but not listed in the table). One can also say that there is no specific factor or group of factors which affects all persons similarly. The relationships of an individual's pulmonary performance to his environment, although interesting, are not thus far

strongly correlated. (Parenthetically, this has been a year of relatively mild air pollution incidence in this area and the challenge to produce any pulmonary responses may have been at the lower limits of adequacy.)

We have also studied serial functional residual capacity measurements in the patient group in relation to their cumulative exposure history to carbon monoxide, total oxidants, temperature, humidity, or other variables. We have found no consistent pattern of rise or fall in the functional residual capacity even with two- or threefold differences in the pollutant exposure history for twenty-four, forty-eight, or seventy-two hours preceding the measurement. In this study at least, we cannot support a dependence of functional residual capacity values upon natural air pollution factors.

In summary, serial studies of pulmonary functions among respiratory patients and among normal humans during the first year indicate: a wide variability in individual test responses from day to day; an "interesting" relationship of the function variability of some of these subjects to multiple factors in the environment; factors to which susceptibility is noted differ for the several subjects; and no specific test in this battery appears as a bellwether of pollutant effect.

PHYSIOLOGIC METHODS FOR MEASURING EFFECTS OF AIR POLLUTION IN MAN

Moderator: G. Filley

The discussion was divided into two parts. The first concerned "Laboratory Methods for Measuring Airway Resistance" and was led by Dr. Arthur DuBois (Philadelphia). Dr. DuBois outlined the plethysmographic method for measuring airway resistance. The subject, in a closed box, alternately compresses and expands the gas in his lungs by panting. The intrathoracic pressure changes are reflected by measurable pressure changes in the air in the box, and a value for "alveolar pressure" can thus be obtained. When this is divided by the mean flow through the airways, a value for resistance can be computed.

Three methods were discussed: the body box or plethysmograph, the esophageal balloon, and the Clements interrupter methods. Dr. DuBois reviewed the basic principles on which the plethysmographic method is based, i.e., Ohm's Law and Boyle's Law. Dr. N. Robert Frank (Boston) has compared the three methods and found that the percentage increase in resistance induced by inhaling sulfur dioxide was related to the method of measurement; thus, the esophageal balloon method yielded the largest and the Clements interrupter the smallest percentage increases. Dr. Frank postulated that the difference might depend on the respiratory frequency at which the measurement was made. Dr. Jay Nadel (San Francisco) showed that volume history must be considered in measurements of airway resistance. After the inhalation of substances which increased airway resistance (sulfur dioxide, cigarette smoke, histamine phosphate, or methacholine chloride aerosol), a single deep inspiration and subsequent relaxed expiration temporarily decreased the airway resistance in normal subjects. He also demonstrated changes in airway resistance after a deep breath using other techniques, including measurement of total lung resistance with the esophageal balloon technique, lung resistance with the ten-cycle interrupter, and maximal rates of airflow measured on a spirometer with a rapid speed kymograph. He cautioned that, since deep inspiration may temporarily change airway resistance, methods that require a prior deep inspiration cannot be expected to give the true values for airway resistance that obtain during normal breathing. Dr. Goetz discussed some physical factors which affect resistance to flow of a compressible fluid; in particular, he believed that airway resistance should rarely, if ever, be considered a constant, i.e., the pressure-flow relationship is dependent on the rate of flow.

Dr. DuBois summarized the physiologic factors which influence the value for airway resistance as follows: rate and direction of airflow, gas density and viscosity, lung volume and lung elastic pressure, antecedent history of lung volume, frequency of respiration, prior administration of bronchodilator or bronchoconstrictor agents (including cigarette smoke) and finally, the degree of generalized airway obstruction which may be estimated provided all of the other factors are controlled. He pointed out that, while acute exposure to some aerosols in high concentration may result in a temporary increase in airway resistance, it is not known whether chronic exposure to lower concentrations produces cumulative or irreversible changes in the patency of the airways.

The discussion of the second main topic of the evening, "Physiologic Tests for the Detection of Chronic Pulmonary Disease in the Field," was led by Dr. Ben Branscomb (Birmingham).

Dr. Howard Dayman (Buffalo), among others, raised the important question whether, even if instrumentation could be made reliable, the meas-

urement of airway resistance is what is needed in field testing. He showed how the expiratory spirogram can be interpreted to throw light on the nature of this resistance. The very first part of an expiration is effort-dependent and the resistance therein is measured for the relatively quiet breathing tested in body plethysmography. By contrast, in the expiratory spirogram, the above introductory phase is followed by outflow having a regulated pattern of deceleration. At a selected position in a vital capacity (after the introductory portion), flow is fixed regardless of the effort applied, but the level at which it is fixed decreases in uniform progression with respect to the volume exhaled. There can be no simple expression for resistance in the portion of the vital capacity which comprises about 80 per cent of the normal spirogram. The simplest expression for the resistive state is the rate at which flow changes with respect to volume change, dV/dV. The value for this relation is remarkably constant for healthy lung served by healthy airways, 2.7, SD ± 0.4, and shows significant depression when the resistive state is increased.

Drs. Branscomb and Robert Hyatt (Beckley, West Virginia) showed expiratory flow data plotted as flow against volume. Although there was slight disagreement regarding the linearity of the expiratory flow-volume relations over the lower 50 to 60 per cent of the vital capacity, all who have been using this method agreed that quantification of expiratory flow in this volume range had the greatest merit. Dr. George Wright made the point that one should not insist that field instrumentation be simple; the ultimate criterion must be adequacy of the instrument to do the job required. He also cautioned against assuming that any one test could detect all cases of chronic pulmonary disease since there are still large gaps in our knowledge of the pathophysiology of these conditions in relation to air pollution.

Abstract from Report of Final Meeting, Wednesday, March 16, 1960, of Joint United States-United Kingdom Board on Cardiorespiratory Disease[a, b]

George R. Meneely

Preface

During the period, March 4–16, 1960, the United States members of the United States–United Kingdom Board visited individuals, hospitals, laboratories, and field research projects in Birmingham, Sheffield, Glasgow, Edinburgh, London, Cardiff, Llandough, and Oxford. These visits led to the formation of certain opinions and tentative conclusions which the United States members believed should be presented to the whole Board. For drafting purposes the subject was divided into topics: terminology, classification, incidence, clues as to differences between the two countries, comments regarding methodology, and studies thought worth while to recommend. Subjects were assigned, such as chronic nonspecific lung disease, cor pulmonale, and arteriosclerotic heart disease. Drafts were corrected and the semifinal versions presented to the whole Board on March 16, 1960. The United Kingdom members of the Board suggested certain changes which substantially clarified the draft. The whole document was generally, although not formally, concurred in by all present.

Terminology

The American visitors were most favorably impressed by the Ciba report (1) and especially by the suggestion that the current confusion could best be resolved by the general adoption of a multi-categorical classification. The following suggestions with respect to such are based on the Ciba report and should be regarded as nothing more than a faltering second step toward the resolution of the existing Tower of Babel situation. Only after numerous suggestions and opinions have been received from both sides of the Atlantic can any generally acceptable nomenclature be derived. And perhaps not then!

Classification of Chronic Nonspecific Lung Disease and of Complicating Cor Pulmonale

I. *Etiologic classification:*

1. Specific infective agents, e.g., tubercle bacillus, fungus, et cetera

[a] From the Radioisotope Center, Vanderbilt University School of Medicine, Nashville 5, Tennessee.

[b] The comments here presented are abstracted from the report which the United States members (Drs. Tinsley Harrison, Harold Dorn, Oglesby Paul, and George Meneely) made to the whole Board. The United Kingdom members were Professors Sir George Pickering, Melville Arnott, C. H. Stuart-Harris, and Donald Reid. While generally concurred in by all present, the report was not formally approved as such but served rather as informational material. Only those parts of the report relevant to pulmonary disease are abstracted here.

2. Allergic
3. Physical or chemical agents, e.g., coal dust, cotton dust, et cetera
4. Fibrotic and infiltrative, e.g., sarcoid, amyloid, collagen disease, et cetera
5. Cardiovascular and renal, e.g., passive congestion, primary pulmonary hypertension, "uremic lung," et cetera
6. Neoplastic, e.g., miliary metastatic carcinomatosis, alveolar carcinoma, et cetera
7. Chronic nonspecific lung disease

(*Note:* The attached morphologic and physiologic classifications refer only to the seventh group above.)

II. *Clinical classification:*
   A. Bronchial asthma
   B. Bronchitis (localized saccular bronchiectasis not included)
      1. *Mucoid*
         (*a*) Simple—without obstruction (includes "smoker's cough" and other mild varieties)
         (*b*) Obstructive
      2. *Purulent* (usually implying destructive changes in bronchi and/or bronchioles, including widespread cylindrical bronchiectasis and/or bronchiolectasis)
         (*a*) Simple—without obstruction
         (*b*) Obstructive
      3. *Accompanying local lung disease*, e.g., localized saccular bronchiectasis
   C. Emphysema (subclassification according to one of the two suggested in the Ciba report)
   D. Cor pulmonale in chronic nonspecific lung disease
      1. Right ventricular hypertrophy without failure
      2. Right ventricular hypertrophy with failure (systemic congestion)

III. Physiologic classification—to be developed

Cor pulmonale in chronic nonspecific lung disease: The Tower of Babel situation was especially pronounced in regard to cor pulmonale and appeared to be intranational (on both sides of the Atlantic) rather than international. Some limit the term to patients with overt right heart failure while others include persons with clear evidence of right ventricular hypertrophy. Still others would include persons with elevated cardiac output due to hypoxia even though the signs of hypertrophy and of congestive failure are both lacking.

It seemed clear that there was no qualitative difference in cor pulmonale between America and Britain. The clinical impression was gained that there was a large quantitative difference, the disorder being more frequent in Britain. However, the confusion which has been mentioned above would appear to throw serious doubt on the accuracy of the available statistics in both countries. If, as we believe, there is a large difference in incidence, it would appear to be due to the greater frequency of chronic nonspecific lung disease in Britain.

Incidence of Chronic Nonspecific Lung Disease

First, as to the kinds of pulmonary disease within the category "chronic nonspecific lung disease" (Ciba Symposium definition) occurring in the United Kingdom, it is the conclusion of the visiting United States clinicians that patients examined in Birmingham, Sheffield, Edinburgh, London, Cardiff, and Oxford all presented clinical pictures which are commonly seen in the United States. This was thought to be especially true of the population of the United States Veterans Administration hospitals and the large municipal hospitals of the states. It was further evident in bedside discussion of these patients that there were no fundamental differences of a conceptual nature between the United States and United Kingdom clinicians, granting that each accepted a lack of understanding of the exact etiologic and pathogenetic processes involved. It was, however, clearly and abundantly evident that there were marked differences in terminology which always caused some coufusion in communication and which at times seemed to disturb or suppress discussion of the factors involved in the particular individual.

On the other hand, it was the distinct although undocumented impression of the United States clinicians that there were differences in incidence and in proportional severity in the occurrence of this group of pulmonary disorders. It appeared to the United States visitors that "chronic nonspecific lung disease" occurred in the United Kingdom with substantially greater frequency than in the United States and, further, that there was a much larger fraction among its victims of those who had severe and prolonged illness resulting from it. This was especially true with regard to the category called chronic bronchitis, many members of which would have been considered by the Americans to have bronchiectasis by reasons of their copious production of purulent sputum. It is at once evident that a new appraisal of the general situation regarding comparative incidence of chronic nonspecific lung disease in the two countries would require more general agreement on terms. The greater frequency and severity of this disorder in the United Kingdom has earned for it a degree of attention it has not received in the States. It was generally the consensus that the morbidity and mortality status of the illness in the United States is very poorly defined.

Clues to differences: The data which have been presented to the visitors have stressed sex, age,

social class, smoking habits, urban versus rural dwelling, and differences in pollution of the atmosphere as important factors in morbidity and mortality from chronic nonspecific lung disease. Age and sex are rather similar phenomena in the two countries. There probably are important differences in the meaning of social class. While the same general social stratification can be developed for both countries, there are quite marked differences between the two as to the significance of class to the individual in terms of standard of living and especially in the extent to which he controls his own and his family's environment.

The United States visitor to the United Kingdom in March soon realized that, while there may not be striking differences in absolute temperature levels between certain northerly parts of the United States and the heavily populated areas of England, there certainly are some important qualitative differences and, more particularly, in the general provisions made for dealing with the extremes of weather. The cold, while not greater in degrees, has a bone-chilling quality most probably due to concurrent dampness. In homes and in places of business there is much less provision for heating. Furthermore, heating is usually provided in an extremely uneven fashion; for example, a warm fire in a living room and little or no heat in bedrooms. There are very real differences between the United States and the United Kingdom in this important matter. The modern American of any but the very lowest economic group lives in an environment which is artificially rendered closely similar to a uniformly temperate climate in which low humidity rather than high is likely to predominate.

The extent and severity of air pollution in the United Kingdom certainly impresses the visitor. It was the general consensus that it would be difficult if not impossible to duplicate scenes of smoke and fog such as were frequently observed during the period of the visit. It does not appear that the air pollution encountered is much different in kind from that seen in the soft-coal-burning cities of the United States, namely, mostly a mixture of carbon particles and oxides of sulfur, but there is a very striking difference in the amount of it and in the amount of associated fogging.

In brief, the most obvious differences between the British environment and the American which would be likely to influence pulmonary disease seem to be in temperature and humidity control of homes and places of work and in a much heavier degree of atmospheric pollution in Britain. It is further probable that cough productive of sputum is accepted and tolerated by the United Kingdom populace and perhaps viewed as inevitable, whereas, among Americans, medical attention would be sought earlier.

In the important matter of smoking, it is quite impossible to relate personal experience to general statistics, and comment on possible differences in this particular are not made. It is evident, however, that this is an area in which further investigation should be undertaken.

Comments Concerning the Testing of Pulmonary Function

United States and United Kingdom workers in clinical pulmonary physiology are generally agreed that pulmonary function is best considered in terms of ventilatory function, alveolocapillary function, and pulmonary circulatory function. In chronic nonspecific lung disease, the first and last of these are probably receiving more attention than the second. Practicable field methods for the evaluation of the second and the third do not seem to exist at the moment. For this reason, principal attention has been directed to ventilatory function. All concerned believed that the time and volume relations of forced expiration offered the easiest and most practical primary function test. The American group thought it highly undesirable to express observations from this test as "indirect maximal breathing capacity" because in tables and graphs the "indirect" often is lost and the reader has the impression that the actual maximal breathing capacity was evaluated, which is not the case. It is generally the consensus that one-second or three-quarter-second volumes (after the first few milliliters) are much superior to peak flow measurements. The latter have been widely used, perhaps because of the existence of a rugged simple instrument for its measurement and the ease with which it can be employed.

The American recommendation is strongly in favor of obtaining the whole vital capacity curve because there are differences of opinion as to which part of the curve may be most revealing of early impairment. The American view favors the volume velocity relations in the last part of the curve.

It is probable that more attention should be given to lung volume measurement and the ratio of residual volume to total lung capacity. This procedure seems of cardinal importance in the study of a disorder characterized in many patients by pulmonary distention. It is possible with many methods to assess the efficacy of intrapulmonary gas mixing which has been shown to be a valuable procedure, especially in prognosis.

A gap certainly exists in field methods for the important alveolocapillary function and the appraisal of pulmonary circulation. Oximeter meth-

ods have seemed attractive in the past but, to date, most of the methods tried have proved unreliable for general use.

The advanced centers in the United Kingdom where research in clinical pulmonary physiology is in progress impressed the visitors. Aside from expressing admiration for the energy and scholarliness with which this work is pursued in the United Kingdom, no comments on this aspect of the subject are offered.

Studies Recommended

To the United States visitors, the following seem to be the most important areas in chronic nonspecific lung disease upon which the immediate research effort should be focused:

Clinical and classical epidemiology: The United Kingdom effort in these areas is advanced beyond that in the United States, e.g., standardized respiratory questionnaire, uniform function studies, and sputum volume measurements. Studies need to be broadened and deepened. The *United States effort* especially *needs stimulation* while the *United Kingdom effort needs continuing and expanding support.*

Morbid anatomic studies: Investigations to date of the lung parenchyma, the bronchi, and the pulmonary circulation have been profitable and need continued and expanded support. Of especial importance is investigation of the interrelations of the manifest bronchial alterations, especially as to the glandular elements and hypersecretion and the destructive parenchymal changes. The blood vessels are beginning to receive the attention deserved, and this research needs to be pressed.

Pulmonary physiology: A threefold effort seems indicated here: fundamental research to relate the clinical picture more closely to the morbid anatomic changes which can be observed; a broadened field effort to characterize the natural history of pulmonary impairment in these disorders; and investigations designed to relate the physiologic alterations observed to pathogenetic factors. Here, of special importance are studies of air pollutants and of the role of infection.

Microbiologic study: It is evident that the role of infection is important but not well understood. Of special importance should be the stimulation of research in the possible role of viruses and continuation of studies of bacterial flora and their effects. The role of childhood pulmonary infections seems especially deserving of study.

Certain areas of collateral interest should be mentioned, notably the *endocrine*, the *immune body* area, *genetics*, and *sociologic* studies.

REFERENCE

(1) Ciba Guest Symposium: Terminology, definitions and classification of chronic pulmonary emphysema and related conditions: A report of the conclusions, Thorax, 1959, *14*, 286.

SESSION V. WHERE DO WE GO FROM HERE?

Moderator: I. Gersh

The final session of the conference consisted principally of a question and answer session. Five of the key questions and the answers to them are presented here.

Question 1. What is the mechanism of plant damage by the air pollutants?

Dr. Haagen-Smit: Synthetic as well as respiratory processes in plants are dependent on the uptake of air. This air is not filtered as in the upper respiratory system of animals, but arrives directly at the cells lining the intercellular spaces through a relatively large opening, the stomata, formed by a few cells on the surface of the leaf. These factors may account for an often greater sensitivity of plants than of animals to toxic materials in the air.

Sulfur dioxide, toxic for men in the 10 to 20 parts per million region, causes severe bleaching and killing of plant cells at concentrations of 0.2 to 0.3 parts per million. Fluorides kill the growing points of the leaves of gladioli at only a few parts per billion, while the maximal industrial levels for fluorides have been set at a few parts per million.

The nature of the effects and the threshold limits for damage vary greatly for the different pollutants, and this fact is used in identifying and tracing emissions. In addition, the same toxic agent causes widely different patterns of damage on different plant species. The damage is also strongly dependent on growing conditions, such as temperature and humidity.

Quite frequently the first directly visible damage appears at the port of entry of the air in the substomatal region; cells become water-logged, and eventually plasmolysis follows. Later the affected cells shrink and disintegrate forming large intercellular spaces. These changes are accompanied by formations of brown oxidation products, disappearance of chlorophyll and deposition of wound cork in adjoining cells.

For the study of the processes leading to these lesions, it is necessary to look at an earlier period

of reversible interference with the normal functioning of the cells. Unfortunately, not much is known about these processes.

From microscopic observations it seems clear that outer membranes of the cells in direct contact with the pollutants change their permeability and, as a result, swelling and waterlogging of the tissues is often observed. What happens after this may be an interference with the proper functioning of some enzyme system. Some of these effects have been studied in the laboratory. For example, the action of fluoride affects phosphorylation steps in photosynthesis. Fluorides as well as other poisons may react with metals involved in enzymic activities. Acid contaminants might interfere with the proper functioning of enzymes and more generally with the behavior of colloidal systems, such as we find in polyvalent electrolytes.

In a number of cases it has been noticed that *growth* of plants is retarded without showing any sign of direct visible damage. These initial reversible stages of damage lend themselves to observation on some of the early interferences with normal plant behavior. This approach is especially promising since growth phenomena in plants have been studied in detail for many decades, and one can study production of growth stimulant as well as the reaction to known quantities of hormone.

The effect of air pollutants on plant growth can be quite large; for example, etiolated oat seedlings grown under controlled temperature and humidity conditions will show growth rates differing by a factor of ten. When the impurities are removed from the air by passing it through carbon filters, such fluctuations completely disappear. For a study of these effects we have to go back to the mechanism of growth of the seedlings. It has been well established that one of the primary growth effects is on the cell wall. This wall is made up of cellulose, pectic substances, and miscellaneous polysaccharides whereby strands of cellulose are held together by pectins. It has been shown that the growth hormone indole acetic acid increases the formation of esterified pectins. This chemical change results in a less rigid structure, which can yield under influence of osmotic pressure, thereby causing cell elongation typical for plant growth. One of the primary effects of the Los Angeles type of smog may well be an interference with this loosening of the structure or an action on the growth hormone itself.

It is interesting to note that these effects are known in corn mutants. In dwarf corn there is a destruction of indole acetic acid by oxidation. Lazy corn got its name because it lost its power to grow upward. Its geotropical response is abolished through a lack of production of the plant hormone.

Such conditions are known in the air pollution field too; the action of ethylene results in a complete upset of geotropic response probably also by interfering with the action of indole acetic acid. The plant therefore cannot respond to growth effects, gravity, and possibly to phototropism.

Early in the sequence of all these reactions may have come the effect on the pectic substances which connect and bind the cellulose and polysaccharide chains together. This primary phase of the reaction of pollutants on the pectins reminds one strongly of a similar conclusion reached by Dr. Dorfman regarding the expansion of animal cells, in which the sulfate mucopolysaccharides may perform the same task in connective tissue as the carboxyl-carrying pectic substances do in plant tissue. These thoughts are, of course, of an exploratory nature only, but I believe they are significant symptoms of a change in attitude toward problems of air pollution damage. We begin to leave behind the descriptive phase of the numerous expressions of damage and start to concentrate on the basic mechanism of action.

I believe that this same restlessness is beginning to develop in the related field of emphysema research, and I am glad to see some of my biochemical colleagues leave the safe heights of description to delve in the unknown, which is the only way to a better understanding and ultimately a cure of the disease.

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Question 2. What are the best epidemiologic methods now available for the detection of chronic bronchitis and obstructive airway disease in a working population?

DR. GILSON: Epidemiology is the study of disease in groups of people in relation to special

causes; in this instance, in relation to atmospheric pollution. Therefore, it requires precise definitions of the disease, of the population studied, and also of the environmental factors. It seems very desirable to see that an appropriate balance is struck between the efforts put into the establishment of the characteristics of the population, the details of the disease, and the pollution measurements. Too many and too detailed a collection of data about either the medical side or the environmental side are likely to add confusion rather than clarification by limiting the inquiry to highly selected groups and places. Criteria we have used for considering indices, both medical and environmental, include the following:

(*1*) Tests must be *simple* to apply. They may require complex apparatus, but they must need the minimal cooperation on the part of the subjects. Tests which can only be done successfully after much practice, i.e., have a large learning effect or require a high level of motivation, are quite unsuitable. We can rarely expect our subjects, who are members of the general public, to return willingly if our apparatus fails, and it is therefore quite essential that its reliability achieve a standard considerably higher than that which is often accepted in the laboratory.

(*2*) The tests must be *reproducible* on the same subject while in the same physiologic state. This must be established before the survey, and it is highly desirable to know also the effect of such things as the time of day, meals, and environmental temperature on the results. Unless all of the tests are to be done by one observer in all groups, it is essential to establish the observer variability in the test due to different observers.

(*3*) The tests must be *sensitive*. That is to say, the variability within the individual must be small compared to the differences expected between population groups exposed and not exposed to the pollutant being investigated. The tests should be *valid*. This is a criterion usually based on *a priori* evidence derived from other sources of information. For example, in a study of the effects of atmospheric pollution by asbestos dust, it might be appropriate to measure lung compliance on account of the diffuse fibrosis in asbestosis; but it would probably not be useful to make this measurement in an investigation of the effect of chronic general atmospheric pollution which is believed to be causing emphysema.

I believe that the plethysmograph has about reached the stage of development mechanically and electrically for it to be used in the field. Our understanding of the variables affecting the results—for example, of airway resistance due to change of lung volume—should make its use more effective. We have used the plethysmograph for such studies in factories while investigating byssinosis. I am less certain that it provides as sensitive an index of change as, for example, the forced expiratory volume. Our experience up to the present suggests that the forced expiratory volume usually shows differences between occupational and other groups, which are significant at a higher level than the measurements of airway resistance. This arises from the poorer reproducibility of the resistance measurement than of the forced expiratory volume.

Question 3. What is the glomus pulmonale?

Dr. Vernon E. Krahl[*]: A new glomus has been discovered just dorsal to the bifurcation of the pulmonary trunk which we have found in the cat, dog, monkey, and man. It is innervated by branches of both right and left vagus nerves. While histologically identical with the other known glomera (jugular, carotid, aortic), the glomus pulmonale is the only one which can monitor fluctuations in the gas content of mixed venous blood. It is conceivable, therefore, that it might take part in the reflex control of respiratory rate, the pulmonary vasculature, and tone of the smooth musculature of the airways. (*See* Krahl, V. E.: The glomus pulmonale: A preliminary report, Bull Sch Med Univ Maryland, 1960, *45*, No. 3.)

Question 4. Is the excessive collapsibility of the larger airways of emphysematous lungs a primary or secondary manifestation of the disease?

Dr. Krahl: During the conference numerous references were made to the degenerative changes which may occur in the bronchial cartilages of chronically diseased lungs. Bronchi of emphysematous lungs may thereby become flimsy tubes which collapse readily upon expiration. It has been observed that cartilages elsewhere in the body, particularly those which cover joint surfaces, survive only so long as they are subjected to the stresses developed in normal joint movement. Dr. Robert Wright has emphasized the functional importance of the elastic framework of the lung—in effect, an elastic continuum—which exerts a circumferential or radial traction upon both the vascular and air-conducting components of the lung. This tractional force is therefore diminished and even locally abolished in emphysema through damage to the elastic fiber framework. I theorize that, as in the case of joint cartilages, so with bronchial

[*] From the Department of Anatomy, University of Maryland School of Medicine, Baltimore, Maryland.

cartilages, subjection to continual stresses may indeed have a salubrious influence upon them. Diminution or cessation of the normal stresses would then permit the degradation of the bronchial cartilages and render the larger airways more vulnerable to compression and collapse.

Question 5. We hear that emphysema has apparently been induced in the horse by manipulation of its bronchial arterial system. Is this true?

COMMANDER DONALD W. EDWARDS, U.S.N.[a]: An investigative project from the Clinical Investigation Center, U. S. Naval Hospital, in collaboration with the University of California School of Veterinary Medicine and supported in part by contract between the Office of Naval Research and the University of California, San Francisco, was set up to classify pulmonary emphysema in the human being. To accomplish this, a precise knowledge of the etiology and pathogenesis of emphysema is required. The clinical evaluation of patients with well-established emphysema has so far been unrewarding and, as pointed out previously at the first Aspen conference on emphysema, may never be capable of shedding light on this subject. Part I of this project consisted of the study of patients with clinical pulmonary emphysema. Part II was an anatomic study set up in a search for an animal having lungs anatomically similar to those of man. The results of this study were presented at the Aspen conference last year by Dr. Richard McLaughlin.

The purpose of this paper is to describe Part III of this project, a pilot experiment designed to test one theory of the etiology and pathogenesis of chronic pulmonary emphysema by an effort to produce the disease in a living animal. The theory selected for investigation is the effect of primary disturbances in the bronchial artery supply to the lungs. The horse, which possesses lungs anatomically similar to those of man and an alveolar distribution of the bronchial arteries like that seen in man, as well as lungs apparently subject to naturally occurring emphysema, was selected as the logical experimental animal.

It was determined by prior investigation in dogs, and subsequently in rabbits, that occlusion of the bronchial arterial system might best be accomplished by the use of intra-arterial chlorpromazine. Walton and McCord found that this drug injected intra-arterially will regularly produce a severe necrotizing obliterative distal endarteritis, followed by intense gangrene. Special equipment and techniques of anesthesia and thoracotomy in horses were developed as required.

From July 1 to November 1, 1959, 7 normal horses were subjected to thoracotomy at the School of Veterinary Medicine. These horses were all male geldings with an average age of six years and an average weight of 1,100 pounds. The bronchial artery of each was injected with amounts of chlorpromazine varying from 400 to 600 mg. in a concentration of 25 mg. per ml. In 4 of these animals it has been possible to observe in sequence the early and long-term effects of chlorpromazine on the lung when injected into the bronchial artery. The following progressive changes have been noted.

At approximately forty hours necrotizing lesions with severe edema, interstitial hemorrhage, and inflammatory reaction were seen in all parenchymal elements. At twelve weeks extensive focal atrophic changes consistent with pulmonary emphysema, including occasional bullous-like lesions, were present. There were markedly enlarged confluent air spaces. The alveolar walls were thickened in some areas, markedly thinned in others, and some were ruptured. Severe sclerotic changes were present in the bronchial arteries, and in some areas obliteration of their lumina were noted. Bronchiolitis was present, some bronchioles being fibrotic and plugged, others partially destroyed but patent. Degeneration of some bronchial cartilage was seen. There was fibrosis of the pleura and intrlobular septa with marked loss and disruption of elastic tissue. In other areas prominent and typical spur formation was noted in the irregularly fibrotic walls of the air spaces, and in sections stained for elastic tissue it was observed that these fibrous spurs contained small amounts of haphazardly arranged tissue which was fragmented and retracted.

The exact mechanisms leading to the occurrences of this phenomenon are as yet not clear. Certainly the prime effect is a result of induced disease in the bronchial artery. Furthermore, it is highly probable that secondary necrosis of all elements represents the pathogenetic mechanism involved. However, without further studies, it is equally possible that emphysema occurred either as a result of obstructive factors secondary to the severe bronchiolitis which was produced or as a result of various auto-immune factors.

In summary, the experimental work to date has been described, and changes in the lungs have been produced by inducing bronchial arterial disease which appears identical with naturally occurring pulmonary emphysema in man and in the horse (figure 8).

[a] From the Thoracic Surgery Branch, U. S. Naval Hospital, Oakland, California; and the University of California School of Veterinary Medicine, Davis, California.

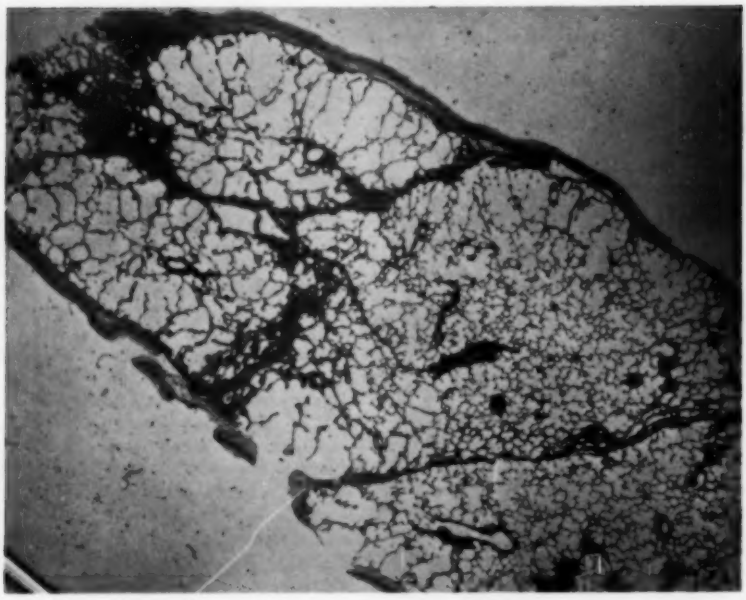

FIG. 8. In this low-power view of horse lung twelve weeks after injection of the bronchial arteries with chlorpromazine the macroscopic changes of emphysema are seen. Note the markedly enlarged confluent airspaces with the walls thickened in areas, thinned in others, and ruptured in others; the fibrotic pleura and septae, the thickened bronchi and bronchioles—some narrowed, some plugged, and some dilated and containing no plugs.

SUMMARY OF CONFERENCE[a]

Julius H. Comroe, Jr.

Aspen is an ideal place for a conference because irresponsible statements by some participants can be attributed to anoxia, and lack of participation by others can be attributed to their shortness of breath at high altitudes, especially in the older, emphysematous group.

A conference can be a very valuable form of communication. Because of the overwhelming quantity and highly technical nature of scientific literature, we all have tended, simply for sheer survival, to become more and more highly specialized: those working on the alveolar membrane, for example, may have excluded from their sphere of knowledge the alveolar capillaries located a few micra on the one side, and surface-active materials a few micra on the other side. This conference has brought together clinicians pathologists, biochemists, biophysicists, geneticists, immunologists, and physiologists (indeed, it has even brought together several groups from England, heretofore somewhat far apart!). It has brought them to the water, though it cannot make them drink.

It is good for *physiologists* to know that the airways are lined with glands and cilia and not with mathematic equations, and that the lungs are made of living elastic and collagen fibers, mucus, enzymes, mast cells, and not of steel springs or rubber balloons.

It is good for the *pathologist* to realize that elegant and informative data can be obtained from living patients or recently dead lungs and that the examination of embalmed

[a] From the Cardiovascular Research Institute, University of California Medical Center, San Francisco 22, California.

bits snipped hither and yon in the lung no longer constitutes the ultimate in scientific knowledge.

It is good for the *biochemist* to be reminded that the chemical composition of a wrist watch—in terms of iron, carbon, nickel, silicon, lead, and radium—is the same whether the mainspring is intact or broken.

It is good for all of us to remember that emphysema is a serious disease, of unknown etiology, of uncertain incidence (though definitely increasing in frequency), and basically not yet susceptible to treatment once the disease is established. Most of us have an almost fanatical zeal (a fanatic being defined as one who redoubles his efforts when he has forgotten his objectives) to solve one small aspect of physiology, anatomy, or biochemistry of the lung and have largely forgotten about emphysema *as a disease*. The fragmentation of medical science into bits and sub-bits has resulted in a spectacular assault on the unknown but has to a certain extent slowed down the very important matter of synthesis of the known. When biophysics followed biochemistry in splitting off from physiology, a frequently asked question was "What is left for physiology?" The answer, at least in part, became evident: that an important role of the physiologist is to put together, in terms of the function of a tissue, an organ, a system or the body as a whole, the important but isolated bits of information coming from the biochemists and biophysicists. (I am told that of 750 scientific investigations reported at a recent national meeting of biochemists, in only two was there study of a whole animal.) Let us see to it that in the study of emphysema some of us have the long-term goal and urgent objective of studying the *disease*.

I would like to make one more general comment regarding communication of science. The conference in the last few years has become an important method of communication, both oral and written; indeed, it is hard for me to find my fellow faculty members because they are usually at a conference or World Congress in Australia, Czechoslovakia, or Argentina. Conference proceedings are rarely published complete with all the scientific data, methods of experimentation, and critical analysis required of an article in a scientific journal, and conference papers are not as a rule subjected to critical evaluation by a board of editors before publication. Because of this, we are in danger of sliding into a new type of scientific communication: "I heard from Dr. X that Dr. Y, who was at the Aspen Conference, heard Dr. Z say that emphysema is caused by eating raw artichokes." Study of printed abstracts may not be much better than the grapevine system; some of these abstracts are indeed quite abstract! I want to emphasize that, in my opinion, your participation here does not relieve you of the responsibility of full scientific publication.

Now for the subject of emphysema. This is indeed a confusing disease. At first thought, we might have common ground and agree that emphysema is a disease of the lung. But even here we are unsure of ourselves because some believe that emphysema of the lung may be only the most obvious aspect of a systemic disease of connective or other tissues. We have not had agreement even at this conference on a definition of pulmonary emphysema. One group here (our morbidity-mortality statisticians) believes that emphysema exists when the word *emphysema* is written on a death certificate by a physician; a subgroup believes such a diagnosis only if the word "emphysema" was written on the death certificate by a clinician educated in or working in a great medical center. This is, to my mind, without meaning. In a large Eastern medical center a few years ago, about 30 per cent of patients diagnosed by the staff as "congestive heart failure" had emphysema as their primary disease and about 25 per cent of the patients labeled "asthma" had emphysema; on the other hand, not infrequently barrel-chested men diagnosed as having emphysema did not in fact have significant emphysema.

Pathologists diagnose emphysema when there is a large, pale lung with larger air spaces and less pulmonary tissue (loss of alveolar septa and capillaries). Physiologists diagnose emphysema when the functional residual capacity and residual volume are increased both absolutely and in relation to total lung capacity, the maximal expiratory flow rate is markedly reduced (far more than the maximal inspiratory flow rate) and is not increased markedly after vigorous bronchodilator therapy, and there is nonuniform distribution of inspired air to the alveoli.

Clearly there is need for clinical-physiologic-pathologic correlation; this will not be easy. I have heard a pathologist chortle with glee because his autopsy diagnosis was quite different from that predicted by the physiologist on the basis of pulmonary function studies in the

living patient. Of course his diagnosis was different; the physiologic studies were performed three and one-half months before the death of the patient. Meaningful correlations must be established by almost simultaneous studies. There is little likelihood of performing physiologic studies on lungs which will be studied by the pathologist shortly thereafter unless (*1*) unilateral studies are done on lungs before and immediately after pneumonectomy, (*2*) physiologic studies are done on lungs in the autopsy room before the histologic studies are begun, or (*3*) pulmonary diseases can be produced in animals. I believe that the pathologist must incorporate certain physiologic studies into his routine and that the clinician must learn to request more physiologic studies and correlate function with currently used clinical and roentgenographic studies. (The latter will be difficult in England because so many of their pulmonary physiologists have settled in the United States and Canada that the English laboratories have returned to the sputum cup for their quantitative measurements!)

Whatever emphysema is, it must be caused by something. If not the end result of inherited weak protoplasm, the disease is probably caused by some defect in the body's defense or repair mechanisms. This conference has given us systematically basic information on the function, defense, and repair mechanisms of each part of the respiratory system: the *nose* and its ability to warm or cool inspired air, filter it, and even soak up certain soluble gases; the *cilia*; respiratory tract mucus, its quantity and qualitative characteristics; the mucoprotein, surface-active material (Clements' substance "X") which coats the alveoli; the alveolar-capillary membranes; the two circulations to the lung; and specific pulmonary tissues and cells.

The conference has touched lightly on the factors determining damage and repair: age? endocrine and metabolic factors? individual susceptibility? The latter, although nebulous, is apt to be important. We know that the blood vessels and heart muscle of some patients can withstand high pressures for many years without failure of either. Similarly, some patients have alveolar distention (asthma) for years without any significant evidence of destruction of alveolar or airway tissue. Dr. Tooley and his associates in our institute have studied thoroughly treated boys and girls who have had severe, repeated asthmatic attacks for five to ten years and have found normal pulmonary function. Does similar stress produce significant damage in older persons or in those whose pulmonary tissues are weakened by some infectious agent? Does "susceptibility" increase with the latter decades of life? Are the maintenance and repair processes less effective with advancing years? How indeed are elastic fibers repaired or replaced at any age?

The reaction of the lung to chemical substances is a fascinating field. Why is the severe, acute pulmonary damage caused by the pneumococcus and by nitric oxide repaired completely, but some types of injury are followed by moderate to extensive fibrosis? Why in emphysema is there an actual *loss* of pulmonary tissue with little or no signs of inflammation and repair? Why are some so-called "virus pneumonias" followed by extensive, permanent tissue destruction?

What is the role of air pollution? Experts have emphasized above all the need for precise information about air pollutants. What did the exposure consist of—vapors or particles? Were the particles hydrophobic or hydrophilic? What was their size(s) and their composition? Were free radicals present even momentarily? We have heard that there is no real chemical composition of smog. Smog is of fantastic complexity; the composition and concentration of its components vary with time, moisture, sunlight, and distance.

What are the mechanisms by which air pollutants may cause emphysema? We have no answers, only conjectures. Direct corrosive action of inhaled chemicals is one mechanism but rare. Interference with the normal protective cleansing mechanisms of the lung may be another, leading to susceptibility to bacteria or viruses. Causation of bronchiolar constriction, inflammation, or increased mucus with resulting obstruction, atelectasis, infection, and destruction is a third possibility. Chromosomal damage and immunologic reactions have been suggested as well.

Is there a single cause of emphysema? Lowell implicated smoking, but all of us have studied patients with the characteristic physiologic and pathologic picture of emphysema who have never smoked or lived in smoke-filled rooms. Asthma and bronchitis are listed in many textbooks of medicine as the forerunners of emphysema, but we know now that these do not necessarily cause emphysema, nor do all emphysematous patients have histories of

asthma and bronchitis. Old age is not necessary for emphysema to develop; some of the aged never develop it, and very severe emphysema may be found in patients between twenty and forty years of age. (It would be worth while to do an intensive study of emphysema in this younger age group with no long history of inhalation of smog or smoke and no pulmonary infection.)

Experimental production of emphysema-like disease in animals will be of value but, if the end picture of emphysema is in fact caused in many ways, there can be no certainty that all human emphysema is produced in the same way. For example, injection of certain sclerosing materials into the bronchial circulation of the horse seems to produce an emphysema-like disease pathologically; yet, in man with emphysema, the bronchial circulation is not only not occluded but may actually enlarge as the pulmonary arterial circulation becomes restricted.

The epidemiologic approach, employed so effectively by the British in the study of silicosis and other diseases, should certainly be used in the study of emphysema, but with the understanding that this approach will not provide absolute proof of etiology but only clues for more definitive experimentation.

Another approach centers on the early diagnosis of emphysema and an intensive and long-term study of these patients: *intensive* with respect to a meticulous history and thorough bacteriologic, biochemical, and immunologic characterization of the individual; and *long-term* with respect to following the progress of the disease and trying to identify associated factors. For example, no one has yet determined whether elastic or elastic-like tissues in the skin, eye, and lungs change independently or concomitantly in any one individual.

I believe there is a need for long-term dedication to the problem. Unfortunately for the study of chronic diseases, much of our research work today is done by research fellows on a one- or two-year term whose careers seem to depend in part on finishing specific research problems and publishing completed work; this results in work on problems that can be completed in one to two years, and the investigation of emphysema as a disease does not fall into this category. I have tried unsuccessfully for years to arouse the interest of younger members of my department in a long-range study of the growth, maintenance, and repair of specific tissues of the lung. Finally, one of them turned on me and said, "If you think it's so important, why don't you do it yourself?" Perhaps I had best end my remarks here.

* * *

Postscript

Ideas for additional research which were discussed at the conference included the following:

1. New methods for the identification and measurement of the air pollutants, since our present methods tend to alter them in various ways.
2. Make direct examination of the behavior and change in character and size of particles after their inhalation into the airways of the experimental animal.
3. The controls of pulmonary blood flow and the role of the glomus pulmonale.
4. The cause of the collapsible airways in emphysema. Are the cartilages responsible? Is the defect due to chronic trauma or to the lack of stress exerted upon them? Does the phenomenon ever precede the disease?
5. The mechanism and significance of polycythemia in chronic pulmonary insufficiency.
6. The mechanism of repair in the various lung tissues.
7. The composition and behavior of healthy lung, i.e., its "chemical anatomy" and the effects of age and disease thereon.
8. Standardization of pathologic nomenclature and descriptions of emphysema.
9. Studies of regional differences in lung function by roentgenographic and isotope methods.
10. Studies of prognosis in chronic pulmonary insufficiency and the prognostic value of lung function tests.
11. The mechanism and development of cor pulmonale in emphysema.

12. Studies of naturally occurring chronic bronchitis, asthma, and emphysema in animals.

13. Continuing efforts to produce these diseases in experimental animals: consider carefully the species used, the physiologic age of the animals, and the possible prior existence or artificial production of some basic tissue defect.

14. Studies of the genetic factor in emphysema, e.g., studies of emphysema in twins.

15. The biochemistry of bronchial mucus and factors influencing its secretion and character. What drugs if any have true expectorant action?

16. Would English pathologists find more bronchitis than American pathologists in American pathologic material? A cooperative study appears to be indicated.

17. The effects of various air pollutants on ciliary action and mucous secretion.

18. Methods to make continuous measurements of the thickness of the mucous layer in the trachea during exposure to various agents.

19. Morphologic studies of respiratory mucous membrane after its ciliary action has been stopped for prolonged periods.

20. The nature of the tissue effects of various pollutants and ways of defending against them.

21. Pressure measurements in all sizes of airways at various phases of respiration.

22. Correlation of morphologic observations on the lungs of man or animals studied physiologically within the previous twenty-four to forty-eight hours.

CHEST CONFERENCE

University of California School of Medicine

CASE UCH 305226

DR. KENNETH MELMON[1]: A 41-year-old man was admitted to the hospital on May 3, 1960, because of wheezing, a productive cough, shortness of breath, and the recent discovery of a hilar mass in the left lung.

History: The patient first had mild wheezing at the age of two. When he was twelve years old, the wheezing became recurrent; at this time he also developed a productive cough and moderate dyspnea, especially on exertion or with respiratory infections. For the past five years these symptoms have been present constantly; during this period sputum volume amounted to about one-half cup daily, and dyspnea increased. For the past two years the patient has had recurrent generalized edema which could be controlled temporarily by diuretic therapy and bed rest.

Physical examination: The patient was a well-developed, plethoric man who was moderately dyspneic at rest. His temperature was 37°C., pulse rate, 80 per minute; respirations, 16 per minute; and blood pressure, 125 systolic, 80 diastolic in mm. of mercury. The lateral expansion of the right hemithorax was one and one-half inches; and of the left, one-half inch; the diaphragmatic excursion was one and one-half inches bilaterally. The entire left hemithorax and the lower two-thirds of the right hemithorax were hyperresonant to percussion, mild expiratory wheezing was present, and breath sounds were greatly decreased. Harsh breath sounds and loud expiratory wheezes were audible over the right upper lobe, and rhonchi could be heard at both lung bases. The area of cardiac dullness could not be defined by percussion. A right ventricular heave was present in the epigastrium; the second component of the basal second sound was accentuated. The liver edge was tender and extended two finger-breadths below the right costal margin. Slight ankle edema was present.

Laboratory findings: The leukocyte count was 9,100 and the hematocrit, 50 per cent. An electrocardiogram showed a right ventricular conduction defect. The results of pulmonary function studies and bronchospirometry are shown in tables 1 and 2.

DIFFERENTIAL DIAGNOSIS

DR. CHARLES T. CARMAN[2]: Dr. Meier, may we see the X-ray films?

DR. CHARLES MEIER[3]: These X-ray films of the chest were taken at the time of admission (figure 1). They show a large density near the superior pole of the left hilum. Radiolucency of the entire left lung is increased, probably due to the pronounced decrease in the size and number of pulmonary vessels. The left hemithorax is somewhat smaller than the right. An old pleural reaction is evident at the base of the left lung and the left diaphragm is slightly elevated; cystic areas are present at the base of each lung and in the right midlung. The left hilar density appears to be a dilatation of the proximal segment of the left pulmonary artery. Subsequent tomograms of the hilar regions showed no encroachment on the trachea or left main bronchus.

This pulmonary angiogram was performed on May 19 (figure 2). It shows that the pulmonary valve and the blood flow to the right upper lobe are normal. The flow to the right lower lung is reduced, and the vessels partially outline the cystic areas in the right lower lung. The proximal portion of the left pulmonary artery is moderately dilated, but the peripheral portion shows a gradual conical constriction. Only a small amount of contrast material is present in the peripheral arterial channels throughout the left lung. Hemodynamic measurements made after angiography showed a right ventricular pressure of 55/5 and a mean right atrial pressure of 10 mm. of mercury.[4]

Bronchograms taken on May 25 (figure 3) show severe fusiform bronchiectasis in virtually all segments of the left lower lobe and slight bronchiectasis in the left upper lobe. The right upper lobe appears normal, but slight cylindrical bronchiectasis is present in the medial and posterior basal segments of the right lower lobe.

DR. CARMAN: Dr. Swenson performed bronchospirometric studies on this patient. Dr. Swenson, would you discuss the pulmonary function and bronchospirometric studies in this case?

DR. EDWARD SWENSON[5]: As shown in table 1, the residual volume was very large, 283 per cent of the predicted value. It accounts for 51 per cent of the total lung capacity. This means that the patient is able to exhale only 49 per cent of his total lung capacity. Such a high ratio of residual volume to total lung capacity reflects severe hyperinflation and is associated with dyspnea in nearly all instances (1). The thoracic gas volume (measured at functional residual capacity in the body plethysmograph) was 6,550 ml., compared with a functional residual capacity (determined simultaneously

[1] Assistant Resident in Medicine.
[2] Assistant Professor of Medicine.
[3] Assistant Professor of Radiology.
[4] Performed by Dr. Malcolm B. McIlroy of the Cardiovascular Research Institute.
[5] Clinical Instructor in Medicine.

TABLE 1

PULMONARY FUNCTION STUDIES*

	Predicted	May 6, 1960	Per Cent of Predicted
Lung volumes			
Vital capacity (ml.)	4,020	3,080	77
Residual volume (ml.)	1,310	3,710	283
Total lung capacity (ml.)	5,330	7,330	138
RV/TLC†	25%	51%	—
Functional residual capacity (N_2 washout) (ml.)	2,320	4,690	202
Simultaneous measurements of			
Thoracic gas volume (FRC) (ml.)	—	6,550	—
Functional residual capacity (N_2 washout) (ml.)	—	4,080	—
Ventilation			
Total volume (rest) (L./min.)	7.5	7.6	—
End expired $P_{A_{CO_2}}$ (mm. Hg)	38–42	42‡	—
Distribution of gas and blood			
N_2 increase (750 - 1,250 ml.)	2.0%	7.7%	—
N_2 after 7 minutes of O_2	2.5%	14.8%	—
Physiologic dead space (ml.)	162	216	—
Arterial blood			
$S_{A_{O_2}}$	96–99%	92%	—
$P_{A_{O_2}}$ (mm. Hg)	95	70	—
$P_{A_{O_2}}$ (after 30 minutes O_2) (mm. Hg)	650	580	—
$P_{A_{CO_2}}$ (mm. Hg)	38–42	48‡	—
pH	7.38–7.42	7.37	—
Mechanics of breathing			
Maximal flow rates (L./min.)			
Expiratory	400	60	—
Inspiratory	300	150	—
Intra-esophageal pressures (cm. H_2O)			
Maximal inspiratory	−30	−5	—

* Performed by Dr. E. Douglas Wigle and Dr. Jerome G. Green of the Cardiovascular Research Institute

† Residual volume/total lung capacity

‡ Measured simultaneously

by the nitrogen washout method) of 4,080 ml. The thoracic gas volume includes all the gas contained in the thorax, while the functional residual capacity includes only the gas volume in communication with the airways (2). The difference between these two values, therefore, means that the chest contained approximately 2 liters of trapped gas, i.e., gas not in communication with the airways.

The greatly elevated values obtained in both the single-breath and seven-minute nitrogen washout tests indicate that large areas of the lung were poorly ventilated—so poorly that even after seven minutes of oxygen breathing the expired nitrogen concentration was still high. The increase in physiologic dead space indicates that a significant number of alveoli were poorly perfused in relation to their ventilation.

The arterial oxygen tension and saturation were low. After thirty minutes of oxygen breathing, however, the oxygen tension rose to a nearly normal level, proving that the primary cause of the patient's hypoxemia was not an anatomic right-to-left shunt in either heart or lungs. The elevated arterial carbon dioxide tension and slightly low pH indicated respiratory acidosis due to alveolar hypoventilation of the lung as a whole.

The mechanics of breathing were also abnormal. Maximal flow rates were sharply reduced, and improved only slightly after administration of a bronchodilator aerosol. The maximal inspiratory

TABLE 2

BRONCHOSPIROMETRIC STUDIES*

	Right Lung		Left Lung	
Per cent of total	55		45	
Measurements, May 1, 1960				
Lung volumes				
Vital capacity (ml.)	1,700	(63%)	1,000	(37%)
Functional residual capacity (N_2 washout) (ml.)	3,130	(61%)	1,990	(39%)
Ventilation				
Minute volume (L./min.)	4.75	(83%)	0.95	(17%)
End expired $P_{A_{CO_2}}$ (mm. Hg)	40	—	24	—
Distribution of gas and blood				
N_2 after 8½ minutes of O_2	6	—	20	—
Gas exchange				
O_2 uptake (ml.)	300	(97%)	10	(3%)
Mechanics of breathing				
Total lung resistance (cm. H_2O/L./sec.)	4	—	20	—
Dynamic pulmonary compliance (slow tidal) (L./cm. H_2O)	0.11	—	0.02	—

* Performed by Dr. Edward Swenson

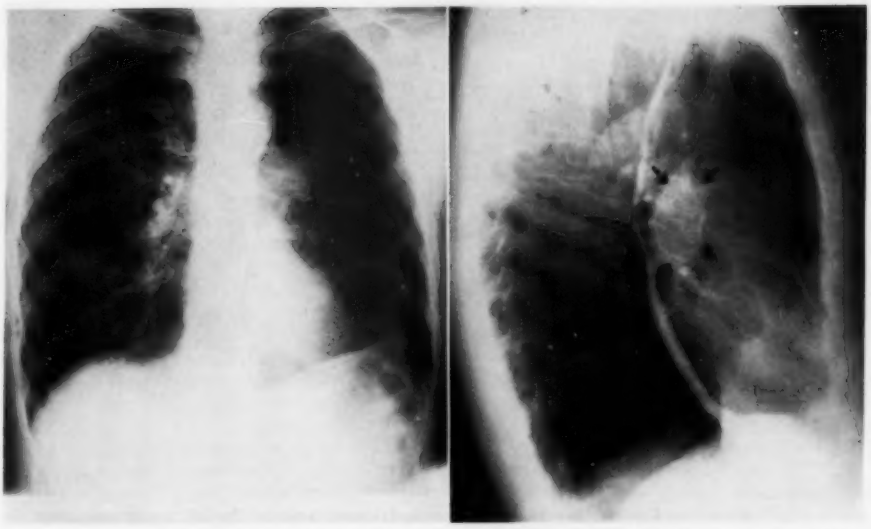

FIG. 1. Roentgenograms of the chest on May 3, 1960, showing hilar density, increased radiolucency, decreased vascular markings, and a small hemithorax on the left.

FIG. 2. Pulmonary angiogram on May 19, 1960, showing proximal dilatation and distal conical narrowing of the left pulmonary artery, decreased vascularity of the left lung and lower portion of right lung.

esophageal pressure was only −5 cm. of water, compared with a predicted value of −30 cm.

Since the roentgenographic abnormalities were predominantly on the left, we performed bronchospirometric studies to determine whether the major physiologic abnormalities were also on the left. As shown in table 2, the contrast between the function of the right and of the left lungs is impressive.

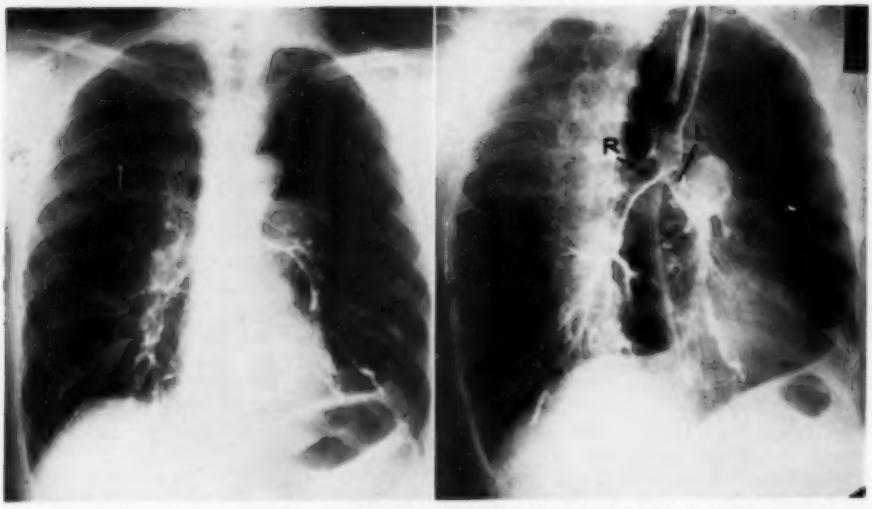

FIG. 3. Bronchograms on May 25, 1960, showing bronchiectasis of the left upper and lower lobes, and medial and posterior segments of the right lower lobe.

The chief abnormalities were in the left lung. Instead of performing 45 per cent of the bilateral function, the left lung accounted for only 3 per cent of the oxygen uptake and 17 per cent of the total ventilation per minute. The high alveolar nitrogen concentration (20 per cent) after eight and one-half minutes of oxygen breathing reflects extreme maldistribution of gas in the left lung. Total lung resistance on the left was increased. If we consider total lung resistance an index of airway resistance, then this increase indicates severe obstructive airway disease on the left. The low dynamic pulmonary compliance reflects decreased distensibility of the left lung; the left lung, in other words, is "stiff."

I would interpret the studies as follows: (*1*) The pulmonary function studies show severe hyperinflation, maldistribution of ventilation and blood flow, alveolar hypoventilation, and airway obstruction. In addition, pulmonary hypertension and respiratory acidosis are present, as well as hypoxemia due to poor distribution of inspired gas. (*2*) The results of bronchospirometry show that, although both lungs are affected, oxygen uptake by the left lung is almost nil. Since it accounts for only 3 per cent of the oxygen uptake, the left lung receives only 3 per cent of the effective pulmonary blood flow. Both the degree of airway obstruction and the decreased distensibility of the left lung are severe. (*3*) The findings suggest a major reduction in blood flow throughout the left lung, probably of long duration. The right lung is involved to a lesser degree.

The findings in the left lung are similar to the experimental results we have obtained in acute unilateral pulmonary arterial occlusion in man and in chronic occlusion in dogs (3). When we occlude one pulmonary artery, oxygen uptake virtually ceases and bronchoconstriction occurs, apparently due to local reduction in carbon dioxide tension in the airways of the unperfused lung. The ventilation, volume, and compliance of that lung are also decreased. Infarction does not occur and the lung remains viable, probably because the bronchial arterial flow increases. In dogs with experimental chronic occlusion, these changes persist for at least three months.

DR. CHARLES T. CARMAN: Confronted by this formidable array of abnormalities, I think our final diagnosis will depend on how we answer the following questions:

1. Is the primary abnormality located in the bronchial airways, in the lung parenchyma, or in the pulmonary vascular bed?
2. Can all the abnormal findings be related to a single disease entity?
3. Are any of the findings incompatible with a single disease?

This 41-year-old man has what amounts to a lifelong history of recurrent wheezing, productive cough, and dyspnea. There was no evidence of an antecedent illness which might account for his

initial symptoms. The symptoms are those of obstructive airway disease, such as occur commonly in chronic bronchitis, bronchiectasis, chronic bronchial asthma, and obstructive pulmonary emphysema, as well as in many other pulmonary diseases. They are certainly nonspecific.

After having had mild to moderate symptoms since the age of two, the patient developed recurrent right ventricular failure at the age of thirty-nine. This disturbing event in the history is the first of two apparent inconsistencies. The mild to moderate symptoms seem insufficient to account for the right ventricular failure. Second, the gross abnormalities found by physical, roentgenographic, and physiologic examination are more severe than the patient's symptoms would suggest. I think that this discrepancy between symptoms and objective findings is an important point in differential diagnosis.

We have abundant evidence that the disease affects the bronchial airways (cough, sputum production, expiratory wheezing, basilar rhonchi, bronchiectasis, gas trapping, reduced flow rates, increased total lung resistance); that it affects the lung parenchyma (bleb formation, reduced dynamic pulmonary compliance); and that it affects the pulmonary vascular bed (right ventricular failure, high right ventricular and right atrial pressure, decreased pulmonary vascular markings, virtual absence of oxygen uptake by the left lung).

Although the patient unquestionably has obstructive airway disease and parenchymal disease, the angiogram demonstrates a striking reduction both in vessel caliber and in blood flow to the entire left lung and lower half of the right lung. Only the circulation to the right upper lobe appears normal. The physiologic studies confirm the severe reduction in blood flow to the left lung. In answer to our first question, I think it is clear that, whatever the disease process may be, the primary lesion is located in the pulmonary vascular bed.

We may now make our second question more specific. What diseases affecting primarily the pulmonary vascular bed could account for all of the abnormal findings? The involvement of multiple structures, the prolonged course of the disease, and the objective findings immediately eliminate many diseases from consideration. The two chief remaining diagnostic possibilities are chronic massive pulmonary arterial thrombosis and hypoplasia of the pulmonary arteries.

Chronic massive thrombosis of the pulmonary arteries is a rare disease which is rarely recognized before autopsy (4–7). It may follow a pulmonary embolus or arise *in situ*, usually in patients with chronic pulmonary or chronic heart disease. The disease is more common in persons in the fifty- to seventy-year age group, but has also been reported in infants and children (7, 8). Clinically, the outstanding features are severe dyspnea and cyanosis (out of proportion to the underlying pulmonary or cardiac disease), syncopal attacks, and progressive right ventricular failure. Routine X-ray films of the chest often show an abnormal hilar density due to pulmonary arterial dilatation proximal to the thrombus, enlargement and distortion of the vessels surrounding the thrombus, reduced peripheral vascular markings, and increased radiolucency of the affected lung (9). Frequently, the site of the obstructing thrombus can be demonstrated by angiography, and cardiac catheterization will often show increased pulmonary arterial, right ventricular, and right atrial pressures (4, 6). Although it is obviously difficult to fix the time of onset, a rapidly progressive course whose total duration is measured in weeks or months is typical of the disease. Occasionally, however, a patient may survive for several years.

Our case shows many of these characteristic features. Nothing in the patient's history, however, suggests either peripheral or pulmonary thromboembolism. He did not have severe dyspnea, cyanosis, or syncopal attacks. Angiography did not demonstrate an acute obstruction, but instead showed a gradual conical narrowing of the left pulmonary artery. Finally, the course of the disease was slowly, rather than rapidly, progressive. I shall therefore reject this diagnosis, recognizing that the lack of any one of these features by itself is not conclusive.

Hypoplasia of the pulmonary arteries is also a rare disease and is probably congenital in origin. Belcher and associates (10), in a report on 13 new cases, reviewed 14 similar cases reported by other writers under such titles as "unilateral pulmonary emphysema" and "abnormal transradiancy of one lung" (11–13). They summarized the important features of this condition in their series. All but one of the patients were males, whose average age was forty-five years. Their symptoms of productive cough and dyspnea were usually mild or moderate and sometimes of long duration. The diagnosis was most often suggested by a typical roentgenographic picture: (*1*) The whole of one pulmonary artery, or one of its lobar branches, was diminished in size, and the segmental arteries were reduced in size and number. (*2*) The radiolucency of the involved lung or lobe was increased, due to the reduced vascularity. (*3*) The affected area of lung was decreased in size. In their series, angiography confirmed the great reduction in the size and number of pulmonary arteries. Bronchography showed bronchial abnormalities in the affected areas in most cases, but these changes were not typical of bron-

chiectasis. The principal functional defects shown by bronchospirometry in their two cases, in which an entire lung was involved, was a greatly lessened oxygen uptake (5 per cent of the total) and tidal volume (13 and 23 per cent of the total).

Obviously, our patient fits the foregoing description in nearly every detail, which provides the answer to our second question. In my opinion, the only disease which could account for all of the abnormal findings is hypoplasia of the pulmonary arteries. The disease involves not only the entire left lung, but the middle and lower lobes of the right lung as well.

Although our diagnostic puzzle now seems completely assembled, we have one small piece left over: the dilatation of the proximal segment of the left pulmonary artery. Dilatation of the affected pulmonary artery has not been a feature of the reported cases of hypoplasia of the pulmonary arteries. It is frequently found, however, in chronic massive thrombosis of the pulmonary arteries.

This brings us to our third question. Is this finding incompatible with our diagnosis? In my opinion, it is not. Dilatation of hypoplastic vessels, with or without associated hypertension, undoubtedly occurs (14). In our patient, the disease is severe, involves four lobes, and probably has been present since birth. Very likely some degree of pulmonary hypertension has been present for a long time. Under these circumstances, dilatation of the proximal segment of the left pulmonary artery is not surprising.

Finally, hypoplasia of the pulmonary arteries is interesting from another physiologic viewpoint. It represents the natural chronic counterpart of the condition Dr. Swenson induces temporarily when he performs unilateral pulmonary arterial occlusion. In fact, it was produced experimentally in man by temporary unilateral occlusion of the pulmonary artery before the naturally occurring disease was recognized.

Dr. Carman's diagnoses: Hypoplasia of pulmonary arteries, left lung, and middle and lower lobes, right lung; bronchiectasis, left lung, and lower lobe, right lung; respiratory acidosis; chronic cor pulmonale and right ventricular failure due to pulmonary hypertension.

Dr. Melmon: After treatment with digitalis, salt restriction, diuretics, and bed rest, the patient's symptoms improved and he was discharged from the hospital. His exercise tolerance remains limited, but he is able to walk one block.

Charles T. Carman, Editor
Mary Morrow, Assistant Editor
University of California School of Medicine
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January 16, 1961

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Notes

A RAPID DRUG-SUSCEPTIBILITY TEST FOR MYCOBACTERIA[1]

Preliminary Report

Many methods are available for determining the susceptibility of tubercle bacilli to various antituberculous drugs; the most widely accepted procedures involve the incorporation of various drugs in one of the routinely used solid or liquid media. Generally speaking, a patient suspected of having tuberculosis is started on one of the accepted regimens of antituberculous chemotherapy almost immediately. Until the bacilli are cultured and the drug susceptibilities determined, however, the physician has little way of knowing the actual drug susceptibility of the patient's infection. The major disadvantage of most drug-susceptibility tests is the length of time required for performance and actual reading of results. Following isolation of tubercle bacilli, it usually takes an additional two to three weeks to determine their drug susceptibility.

Over the years a number of publications have appeared in which various oxidation-reduction indicators, such as methylene blue and tetrazolium salts, were used to demonstrate culture viability or metabolic activity.[2-4] Several investigators also have applied this technique to the development of relatively rapid drug-susceptibility tests.[5-8] Recently, two papers have been published[9, 10] which describe relatively rapid tests in which the metabolic activity of the organism was indicated through use of the oxidation-reduction indicator, resazurin. In an attempt to adapt the foregoing procedures to a more rapid drug-susceptibility test, the present study involved modifications of the procedure described by both Pital and associates[9] and Sorensen.[10]

The *preparation of varying drug dilutions* was arrived at in two ways. The simplest procedure was the use of one of the drug-susceptibility disc sets[11] available commercially. The other procedure employed was to prepare stock solutions of the various antituberculous drugs in distilled water, sterilize the solutions by Seitz filtration, and dilute them to usable concentrations in sterile distilled water. These "usable concentrations" were then dispensed in 0.1-ml. amounts into sterile screw-cap tubes to which the inoculum of organisms was later added.

An 0.05 per cent aqueous solution of the oxidation-reduction indicator resazurin was prepared by adding the desired amount of resazurin to previously boiled water. The resulting bluish-purple solution was stable for weeks at room temperature.

The *inoculum of organisms* was prepared in one of two ways. When the culture first appeared on the isolation medium it was transferred to Tween®-albumin broth and incubated for seven days at 37°C. to obtain a well-dispersed culture. The alternate procedure required that several colonies of acid-fast bacilli, picked from the isolation medium, be carefully ground up in a few milliliters of sterile saline; the resulting suspension was allowed to stand so that the larger clumps settled out, leaving the more homogeneous supernatant suspension for use in the test. In both of the above cases, the culture suspension was further diluted tenfold or one hundred fold in Tween-albumin broth to prepare a "working inoculum" for use in the test. This dilution was such that the inoculum was just barely turbid.

The *actual test* was performed in the following manner. The various drugs, as needed, were added to a series of sterile screw-cap tubes (15 × 125 mm.), using either drug discs or sterile aqueous solutions of the drug. The various drugs used, together with recommended concentrations, have been described previously.[12, 13] One milliliter of

[1] Presented in part before the Medical Session, as part of *Section 4B*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 17, 1960.

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TABLE 1

COMPARISON OF DRUG SUSCEPTIBILITIES BY ROUTINE AND RAPID METHODS

Culture	Method	Control	Isoniazid				PAS			Streptomycin	
			0.2	1	5	10	1	10	100	10	100
H37Rv: 2 strains	Routine	+	−	−	−	−	−	−	−	−	−
	Rapid	+	−	−	−	−	−	−	−	−	−
Isoniazid-resistant, human: 3 strains	Routine	+	+	+	+	+	−	−	−	−	−
	Rapid	+	+	+	+	+	−	−	−	−	−
Avian: 2 strains	Routine	+	+	+	+	+	+	+	−	−	−
	Rapid	+	+	+	+	+	+	+	−	−	−
Battey	Routine	+	+	+	+	±	+	+	+	+	−
	Rapid	+	+	+	+	+	+	+	+	−	−
T-128	Routine	+	+	+	+	+	+	+	+	+	+
	Rapid	+	+	+	+	+	+	+	+	+	+
Saprophyte	Routine	+	+	+	+	+	+	+	+	+	±
	Rapid	+	±	+	+	+	+	+	+	+	+
T-126: 2 strains	Routine	+	+	+	−	−	+	+	+	+	±
	Rapid	+	+	+	+	+	+	+	+	+	±

+ = Active metabolism, hence recorded as drug resistance
− = No detectable metabolism, hence recorded as drug susceptible
± = Partial resistance

the bacterial suspension was added to each drug tube and to one nondrug-containing control tube. All of the tubes were placed in the incubator at 37°C. One or two days later, one drop of resazurin solution was added to all of the tubes and they were replaced in the incubator. When the control tubes turned pink, indicating active bacillary metabolism, the drug tubes were observed. A pink or red color was interpreted as indicating resistance to the drug, a purple coloration as partial resistance, and a blue tint as drug susceptibility. It was found best to read drug-containing tubes within one or two days after the controls had changed because prolonged incubation resulted in drug-susceptibility cultures giving the false appearance of drug resistance. In the case of rapid-growing acid-fast bacilli, e.g., unclassified *Group IV* and *M. fortuitum*, the resazurin was added at the same time as the bacterial inoculum. In such cases the results were available within six to eight hours or less.

A representative comparison of the rapid technique with one of the routine drug-susceptibility procedures is shown in table 1. The routine procedure used for comparison is described in a previous publication.[12] Although only a few examples are shown, the rapid-test procedure has been repeated fifty-five times on a total of 21 different cultures in this laboratory. Using a somewhat different technique, but employing resazurin to indicate culture viability in the presence of serum containing isoniazid, Knight and co-workers[14] have successfully tested their procedure on thirty-five different occasions.

A perusal of table 1 shows good correlative results between the rapid and routine methods. There are two instances in the table (*see* cultures T-126 and Battey) wherein the routine and rapid tests do not agree. In the case of culture T-126, this may be due to the phenomenon described by both Koch-Weser and Ebert[6] and by Pital and associates[8] in which multiplication of tubercle bacilli may continue in the presence of isoniazid for four to six days before drug activity is manifested. If this is the case, then perhaps time and a study of larger numbers of strains may reveal that cultures exposed to isoniazid will have to be left in contact with the drug for four days before the addition of resazurin. It should be pointed out, however, that in the experience of this laboratory such a degree of variance as described above may be detected when repeated drug-susceptibility tests are performed on the same culture, by the same or different persons, using the routine test alone.

On the basis of this study, it is suggested that

[14] KNIGHT, R. A.: Personal communication.

the drug-susceptibility test employing resazurin to indicate culture viability might find application in many clinical laboratories where drug susceptibilities are routinely tested. The advantages of this technique are speed (results in three to five days) and the need for only one medium to which the representative drug discs or drug dilutions may be added as needed.

In summary: A relatively rapid drug-susceptibility test for tubercle bacilli is described. Broth suspensions of the microorganisms were incubated in the presence of various antituberculous drugs for one or two days, at which time one drop of resazurin indicator solution was added to the tubes. Viable and, hence, drug-resistant organisms turned the indicator pink or red, whereas drug-susceptible organisms did not change the original blue color of the resazurin solution.

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FREQUENCY AND EPIDEMIOLOGIC SIGNIFICANCE OF UNCLASSIFIED MYCOBACTERIA IN MEXICO

The isolation of acid-fast bacilli, other than tubercle bacilli from clinical material, has received growing attention since it was shown that a number of these microorganisms might be the etiologic agents of tuberculosis-like diseases.[1, 2] Unclassified mycobacteria are widely distributed throughout the world. Nevertheless, the frequency with which they are isolated is extremely variable.[3] Frequency differences may be due to: (*a*) uneven geographic distribution, (*b*) type of specimens studied, i.e., sputum, gastric contents, et cetera, and, (*c*) the fact that probably some writers consider only certain strains as unclassified mycobacteria, while other strains are viewed as unidentified saprophytes. In the present paper, the term "unclassified" mycobacteria will be used to designate all mycobacterial strains that are not *Mycobacterium tuberculosis* or *Mycobacterium bovis*. That term includes, for purposes of convenience, strains that could eventually be classified as rapid growers, i.e., *Mycobacterium smegmatis, Mycobacterium phlei*, and *Mycobacterium fortuitum*. The term may also include *Mycobacterium avium*, since this species cannot be differentiated with the methods used at present from the nonphotochromogens.[4]

Some writers[5–8] claim that antituberculous chemotherapy somehow increases the frequency of isolation of chromogenic mycobacteria; this belief, however, is not generally accepted.[9–11]

The present paper is a report of the distribution of unclassified mycobacteria in Mexico. It includes surveys made in Acapulco, Mexico (a sea-level town situated in the southeast area of the country) and in the City of Mexico, situated in the central region of the country at 7,000 ft. above sea level. The specimens studied were collected from children and adults suspected of having tuberculosis and from a group of nontuberculous patients with other pulmonary disorders such as emphysema.

The material studied consisted of 3,315 specimens distributed as follows: 2,437 sputum specimens were obtained from patients with tuberculosis which had been diagnosed in previous studies; these patients were hospitalized in the tuberculosis wards of the Pneumology Unit, General Hospital, Mexico City. Sixty-five specimens were collected from nontuberculous patients suffering from chronic bronchitis, most of them with pulmonary emphysema; these patients were hospitalized in the bronchitis ward of the above mentioned unit. Eight hundred and thirteen specimens (352 sputum specimens and 461 gastric aspirates) were obtained from persons with pulmonary lesions evidenced roentgenographically during a pilot project on ambulatory chemotherapy carried out in Acapulco by the Mexican Campaign Against Tuberculosis.

Processing of specimens: All specimens were homogenized with 4 per cent sodium hydroxide, neutralized with 5 per cent hydrochloric acid, and 0.2 ml. of the sediment was inoculated into each of two tubes of Löwenstein-Jensen medium; subsequently, the strains were subjected to the following determinations:

(*a*) Niacin test: Cultures with morphologic characteristics resembling those of tubercle bacilli and giving a positive reaction to niacin were considered to be *M. tuberculosis*. The niacin test first described by Konno[12] was carried out on all strains according to the improved technique of Gutiérrez-Vázquez.[13] When more than one strain was obtained from a patient, the niacin test was carried out on each strain isolated.

(*b*) Inoculation of laboratory animals: When the niacin test was negative and the colonial morphology and the time of appearance of growth suggested the presence of bovine tubercle bacilli, guinea pigs and rabbits were inoculated with 0.02 mg. wet weight of bacilli. Stains displaying high pathogenicity for both guinea pigs and rabbits were considered to be *M. bovis*.

(*c*) Slide cultures: If the colonial morphology was suggestive of Nocardia, slide cultures were carried out on Bennett-agar to observe the characteristic filamentation, branching, and fragmentation of members of the Nocardia species.

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TABLE 1

FREQUENCY OF ISOLATION OF UNCLASSIFIED MYCOBACTERIA FROM HUMAN SOURCES IN MEXICO

	Number	Per Cent
Total specimens	3,315	100
Total positive cultures (tubercle bacilli plus unclassified mycobacteria)	768	23.1
Positive unclassified cultures (per cent with regard to the total of specimens)	160	4.8
Positive unclassified cultures (per cent with regard to positive cultures)	160	20.8

Grouping of unclassified mycobacteria: Colonies without the above-mentioned characteristics were considered to be unclassified mycobacteria. In order to group these microorganisms, the practical classification proposed by Runyon[14] was used. The group-defining characteristics are the following:

Group I, photochromogens. They produce bright yellow colonies when exposed to light. The colonies are colorless when the cultures are incubated under darkness.

Group II, scotochromogens. Strains belonging to this group are strong producers of an orange pigment when grown in the presence of light as well as in the dark. A yellow-orange pigmented sediment is produced when the strains are allowed to grow in Proskauer and Beck medium.

Group III, nonphotochromogens. These microorganisms usually form nonpigmented or slightly yellow colonies which are not affected by light. These are the so-called Battey type of strains.

Group IV, rapid growers. In this group are included strains which grow from small inocula in one to three days in Löwenstein-Jensen medium. This is in contrast to the slower rate of growth of strains of the preceding groups, which grow well after seven to twelve days of incubation. *Group IV* comprises strains both pigmented and nonpigmented. However, it is their growth rate and not the pigment that differentiates this group from the others.

From a total of 3,315 specimens studied at this laboratory, 160 strains of unclassified mycobacteria (4.8 per cent) were isolated (table 1). The grouping of these strains according to Runyon's classification is given in table 2.

Fifteen additional strains were received from the Children's Hospital at Mexico City. They were classified as shown in table 3. From this last group subcultured strains only were received. Therefore, the total number of specimens studied and their incidence in hospitalized children is not known.

Frequency distributions of isolated unclassified strains in relation to the source of the specimen are given in table 3. Most of the unclassified strains isolated were identified as rapid growers (*Group IV*). The second in order of decreasing frequency were the scotochromogens, followed by the nonphotochromogens. The failure to isolate photochromogenic strains is noteworthy and could indicate very low incidence or absence in the population groups subjected to study.

Although the role of unclassified mycobacteria, as probable disease-causing agents, is not discussed here, it would be worth while to mention that very few strains were apparently related to pathologic processes. Three rapidly growing strains were isolated from children with pulmonary lesions, while another rapidly growing strain was isolated from the cerebrospinal fluid of a child. One scotochromogenic strain was isolated from an abscess of the spinal cord, while two other scotochromogens were isolated from two children with cervical adenitis. Some nonphotochromogenic strains appeared to be related to the pulmonary lesions of three adults.

It is widely accepted that nonphotochromogenic bacilli (Battey strains) are usually associated with severe lesions in the lungs. In this study they have been found either alone or with tubercle bacilli, but in the majority of cases they were not the primary cause of the lesions. It is possible that they are different from the so-called Battey strains. At the present time, however, there is no available data that would provide a basis for their differentiation.

The incidence of *M. tuberculosis*, *M. bovis*, and of the Nocardia species will be reported separately.

* * *

It is actually impossible to decide if chromogenic bacilli can arise in tuberculous patients as a consequence of prolonged chemotherapy. This hypothesis is based on the frequency of isolation of

TABLE 2

GROUPING OF THE UNCLASSIFIED MYCOBACTERIA ISOLATED

	Isolated in Pure Culture	Isolated Joined with Tubercle Bacilli	Unclassified Strains	
			Number	Per Cent
Photochromogens	0	0	0	0
Scotochromogens	19	15	34	21.2
Nonphotochromogens	11	12	23	14.4
Rapid growers	59	44	103	64.4
Totals	89	71	160	100

[14] RUNYON, E.: Amer Rev Tuberc, 1955, *72*, 866.

TABLE 3

DISTRIBUTION OF UNCLASSIFIED MYCOBACTERIA ACCORDING TO THE TYPE OF SPECIMEN FROM WHERE ISOLATED

Specimen	Number of Specimens	Number of Cultures Positive for Tubercle Bacilli	Photochromogens		Scotochromogens		Nonphotochromogens		Rapid Growers	
			Number	Per Cent*	Number	Per Cent	Number	Per Cent	Number	Per Cent
Tuberculous Patients, General Hospital, México, D.F.										
Sputum	2,437	457	0	0	22	0.9	16	0.65	63	2.5
Bronchitic, Nontuberculous Patients, General Hospital, México, D.F.										
Sputum	65	0	0	0	3	4.6	0	0	4	6.1
Patients with Pulmonary Lesions Seen on Roentgenograms from Acapulco, México										
Sputum	352	124	0	0	4	1.1	5	1.4	18	5.6
Gastric contents	461	98	0	0	2	0.5	3	0.6	20	4.9
Strains Isolated from Children, Children's Hospital, México, D.F.										
Bronchial aspiration			0		2		1		6	
Gastric contents			0		0		0		3	
Cerebrospinal fluid			0		0		0		1	
Miscellaneous			0		2		0		0	

* The percentages of unclassified mycobacteria are based on the total number of specimens studied

chromogenic bacilli from treated tuberculous patients and on the scarcity of isolation from untreated patients. Thus, Burrows and Barclay[9] have isolated chromogenic mycobacteria from the gastric contents of only one of 400 nontuberculous patients; those writers have concluded that "the frequent isolation of such organisms in tuberculosis sanatoriums would seem related in some way to tuberculous infection or to the chemotherapy thereof; the data suggest that these organisms are infrequent, incidental saprophytes in the population as a whole." This is not conclusive, however, since no comparative data were recorded from tuberculous patients in that particular area.

Comparing the frequency of isolation of unclassified mycobacteria from the gastric contents of healthy persons and from patients with pulmonary disorders, Atwell and Pratt[11] have found that the frequency in both groups of patients was very similar. Those writers concluded that their data would suggest a sporadic, inconstant source of unclassified mycobacteria unrelated to tuberculosis or chemotherapy, and found no evidence to support the theory that these organisms are variants of *M. tuberculosis*. In a study of 122 specimens from healthy adults, Edwards and Palmer[15] encountered 17 strains (14.7 per cent) of unclassified mycobacteria.

Tarshis[5, 6] has noticed an apparent increase of chromogenic acid-fast bacilli in drug-treated patients as compared with that in healthy persons. Although this appears to be true, it is not conclusive evidence from which to assume that drugs have induced such variations of *M. tuberculosis* as to obtain chromogenic descendants. Coexistence of two phenomena does not necessarily imply a cause and effect relationship. In the present survey, a relatively high frequency of scotochromogenic mycobacteria was found in patients who were nontuberculous but had distinct pulmonary disorders such as chronic bronchitis. These patients were subjected to prolonged chemotherapy with chloramphenicol and oxytetracycline, or drugs with a broader antimicrobial spectrum than dihydrostreptomycin. This fact may be similar to the phenomena observed with other microorganisms. For instance, it is not common to isolate *Candida* sp. from healthy persons, while its incidence in drug-treated tuberculous patients is approximately 20 per cent[16]; when patients are treated with wide-spectrum antimicrobials, the incidence of Candida

[15] EDWARDS, L. B., AND PALMER, C. E.: Amer Rev Resp Dis, 1959, *80*, 747.

[16] GONZÁLEZ-MENDOZA, A., AND BOJALIL, L. F.: Amer Rev Tuberc, 1958, 77, 543.

can be increased up to 40 per cent.[17, 18] The role of the antimicrobial drug here appears to be one of selecting those microorganisms that are resistant to it. The same could be true in the case of chromogenic mycobacteria. This would explain the relative high frequency of these organisms in drug-treated patients, whether tuberculous or nontuberculous.

In summary: A survey was made of the frequency of unclassified mycobacteria in Mexico. The highest number of strains was found to be the rapid growers followed in order of decreasing frequency by the scotochromogens and finally by the nonphotochromogens. Photochromogenic strains were not isolated from a total of 3,315 specimens. Furthermore, none of the 15 additional strains of mycobacteria isolated from children which were received at this laboratory for proper classification were identified as photochromogens.

The frequency of isolation of rapidly growing acid-fast bacilli was the same in sputum and in gastric contents. On the other hand, scotochromogens as well as nonphotochromogens were more frequently isolated from sputum than from gastric contents. Very few of the 175 strains of unclassified mycobacteria studied were apparently related to pathologic processes.

In view of the relative high incidence of chromogenic mycobacteria in drug-treated patients, whether tuberculous or nontuberculous, it is suggested that unclassified mycobacteria are more likely to arise as a result of rupture in the biologic equilibrium of the microflora conditioned by chemotherapy than as variations of typical tubercle bacilli induced by chemotherapeutic agents.

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October 8, 1960

[17] Young, G., *et al.*: J Bact, 1956, *72*, 525.
[18] Helms, P.: J Clin Path, 1956, *9*, 372.

Correspondence

ON TARSHIS' MICRO-NIACIN TEST FOR DETECTING HUMAN TUBERCLE BACILLI

To the Editor of the American Review of Respiratory Diseases:

I read with interest the work on "A Direct Micro-Niacin Test for Differentiating Human Tubercle Bacilli From Other Mycobacteria," by Maurice S. Tarshis, which appeared in the July, 1960, issue of the REVIEW (volume 82, page 82). This paper suggests a modification of a technique previously described for the same purpose by the present writer.[1, 2] It is surprising, therefore, that Tarshis makes no reference at all to those papers, one of which was published almost two years ago, while the other appeared in the REVIEW in 1960.

In my 1958 paper, the spot-test method for the differentiation of human tubercle bacilli from other mycobacteria was described as the result of a search for a micro-chemical method to provide consistent and reproducible results when carrying out such differentiation; the method is based on preparing water extracts of cultures grown on Löwenstein-Jensen medium, and testing such extracts for niacin with *o*-tolidine and cyanogen bromide. The reaction is performed on porcelain spot-test plates. A summary of this paper appeared in *Biological Abstracts* (October 22, 1959, *34*, abstract No. 8097). In my second paper, a report was presented on 80 additional strains which were studied by the spot-test method, and the results were consistent with those reported in the previous paper; in addition, it was proved that the method can be carried out with old stored cultures, and that the results of the test are free from the effects of pH.

On the other hand, the core of Tarshis' paper is also a micro-test for the same purpose; it is based on obtaining similar water extracts from cultures, and testing them for niacin on porcelain spot-test plates. In the introduction of his paper, Tarshis mentions three objectives for undertaking his task. Of these three objectives, two were accomplished two years before,[1] namely, whether autoclaved water extracts of the cultures could be used instead of viable culture material, and whether smaller amounts of the reagents could be used without significantly affecting the sensitivity of the procedure.

In the present writer's opinion it seems important that a journal with the prestige of the REVIEW, whose norms for the acceptance of manuscripts seem to be sufficiently strict, should publish a paper like Tarshis', in which the author makes no reference at all to a direct antecedent of his work; this is particularly surprising in view of the fact that the same journal had previously published one of my papers on the spot-test method.[2]

I am taking the liberty of calling your attention to this matter with the hope that the standards of the REVIEW will be maintained so as to avoid errors of this type.

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September 28, 1960

[1] GUTIÉRREZ-VÁZQUEZ, J. M.: Rev Latinoamer Microbiol, 1958, *1*, 287.

[2] GUTIÉRREZ-VÁZQUEZ, J. M.: Amer Rev Resp Dis, 1960, *81*, 412.

STUDY OF LUNG SPECIMENS PREPARED BY FUME FIXATION

To the Editor of the American Review of Respiratory Diseases:

Reference is made to the article, "Study of Lung Specimens Prepared by Fume Fixation," by Elise Jones, in the November, 1960, issue of the REVIEW (volume 82, page 704).

We have experienced no difficulty in preparing fume-fixed specimens for microscopic study that show atelectasis, emphysema, and apparently normal-sized alveoli within the same section. Distended alveolar spaces have occasionally been observed, but these areas are minimal and are frequently located in the subpleural regions. We feel that air supply should be cut to a minimal trickle and the lung permitted to inflate slowly, with fissure approximation occurring in approximately twenty to thirty minutes before adjusting final air pressure. All of our lung specimens, with the exception of large neoplastic nodules, have fixed in excellent condition. Perhaps this is due to the fact that we have a separate air compressor which is not hooked up to the hospital air supply and, therefore, is not subject to excess fluctuations in the reserve tank. These differences in technique might well explain the differences in results.

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December 23, 1960

EXPERIMENTAL TUBERCULOUS CAVITIES AND EMPHYSEMATOUS BULLAE

To the Editor of the American Review of Respiratory Diseases:

The recent article by Drs. Yesner, Bernstein, and D'Esopo on the evolutions of bullous cavities in tuberculosis[1] is a provocative study. I should like to take issue with them on their interpretation of our previous work.[2] Regarding terminology, Dr. Yesner and his associates purposely use the noncommittal term "pneumocyst" and the controversial phrase "bullous tuberculous cavity" rather interchangeably. We believe the evanescent inflammatory pneumocyst, i.e., developing in staphylococcal infection in the more delicate child's lung, to be a different entity from the characteristic tuberculous cavity.

Our experimental preparations showed two distinct types of pulmonary lesions: the central, thick-walled, necrotic cavity; and the peripheral, subpleural, thin-walled, clean vesicle or bullae. The counterpart of these lesions in the thick pleura of human lung has been recognized for many years. Dr. Medlar[3] observed obstructive emphysema associated with fibrotic scars in both experimental tuberculosis in the rabbit and human necropsy specimens, although he concluded that tuberculosis was apparently a rare etiologic factor in bullae formation. Occasionally these bullae become large enough to require surgical resection.[4] More commonly, these lesions in humans are localized vesicular or bullous emphysema in the marginal subpleural areas of focal parenchymal scarring.

We attempted to show representative photomicrographs demonstrating necrotic material in the lumina of the bronchioles as well as an active bronchiolitis which further compromised the lumen. These findings were consistent in nearly 500 serial sections. There is no disagreement about the check-valve mechanism, at least in the acute stage. Temporary and often reversible obstructive emphysema in which inflammatory products and edema supplement organic fibrosis may be seen in exacerbations of asthma, bronchitis, and similar diffuse airway disease.

It is our concept that pulmonary cysts, tuberculous cavities, and emphysematous blebs are three distinct pathologic lesions. It would seem that distortion of bronchi could occur in many inflammatory states, creating transient anatomic flap valves. Expiratory resistance would appear to be dependent upon incomplete and possibly fluctuating degrees of obstruction.

Our impression of the illustrations of Dr. Yesner and co-workers is that their findings were similar to our own. They clearly showed two lesions: a thin-walled, peripheral, subpleural bleb—an emphysematous phenomenon; and a deeper, subcortical, necrotic, thicker-walled tuberculous cavity. Certainly, cavities may enlarge with excavation of necrotic material via distorted bronchi to become giant tension cavities. However, in this instance the pathogenesis of the giant tuberculous cavity would appear distinct from the development of emphysematous blebs or bullae.

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December 27, 1960

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[1] YESNER, R., *et al.*: Amer Rev Resp Dis, 1960, *82*, 810.
[2] BELL, J. W.: Amer Rev Tuberc, 1958, *78*, 848.
[3] MEDLAR, E. M.: Amer Rev Tuberc, 1955, *71* (Supplement, p. 44).
[4] SHEK, J. L., *et al.*: J Thor Surg, 1956, *32*, 96.

Books

On Tuberculous Eye Diseases in BCG Vaccinated Individuals. By Pentti Miettinen. Pp. 126, Ejnar Munksgaard, Copenhagen, 1958. Price, 2500 dan. kr.

Dr. Miettinen has written a very well-documented monograph on tuberculous eye diseases in BCG-vaccinated persons. His material was collected at the Ophthalmic Clinic of the University of Helsinki from 1942 to 1956. It included a total of 1,026 patients with tuberculous or probably tuberculous eye disease. One hundred and forty-nine (15 per cent) of the patients were BCG vaccinated. Of these, 73 (49 per cent) belonged to the group of tuberculous, and 76 (51 per cent) to that of probably tuberculous eye diseases. The BCG-vaccinated cases were distributed as follows on the basis of the ophthalmic disease:

Phlyctenular keratoconjunctivitis	62
Scleritis	1
Sclerokeratitis	7
Retinal periphlebitis	2
Iridocyclitis	42
Choroiditis	11
Uveitis	16
Keratitis	8

Dr. Miettinen states that many investigators still believe that Werdenberg's Law applies to all of the different forms of tuberculous eye diseases. According to this law, slight lung changes are found in severe tuberculosis of the eye: all of the eye symptoms are benign and transient. Prigozhina (1955) made a thorough pathologico-anatomic study of the subject and, of his sample total of 98 eyes examined, every third case with manifestations of primary tuberculosis displayed ocular changes. In disseminated forms of tuberculosis, changes were demonstrable in every second case. In fibrocavernous tuberculosis, changes in the eye were rare and nonspecific.

Phlyctenular keratoconjunctivitis in children has become a relatively common finding. The majority of these patients are tuberculin positive. It is a fundamental rule that the basic allergy is tuberculous and the phlyctenular reaction is nonspecific.

In diseases of the uvea, two different tuberculous uveites must be distinguished. Typical uveal tuberculosis is caused by bacillar metastases; it occurs as do other forms of extrapulmonary tuberculosis in childhood, and is unilateral by nature. The atypical form, on the other hand, is due to the remote effect of the tuberculous focus. Tuberculous iridocyclitis is regarded as a metastatic disease, the bacilli being disseminated by the blood stream.

Tuberculous choroiditis is more common in adults than in children. It is more common in women than in men, and generally occurs bilaterally. Disseminated tuberculous choroiditis is usually attributed to tuberculous bacillemia.

Retinal periphlebitis is a chronic disease, and usually occurs in men between the ages of twenty and thirty-five.

Tuberculosis of the cornea is regarded as endogenous in origin and is often accompanied by inflammation of the ciliary body, iris, and sclera.

Tuberculous deep scleritis is mostly endogenous and is caused by bacterial metastases. Miettinen states that it was Arvid Wallgren who introduced the intradermal method of BCG vaccination in 1927. Vaccination was started in Finland in 1941, when about 1,800 people were vaccinated. A person is considered to have achieved immunity when he has become tuberculin positive, and immunity continues so long as the sensitivity to tuberculin continues. It is stated by Miettinen that tuberculosis in children is rare today and that no notable increase in the incidence of the disease in boys and girls occurs until after the age of puberty. In men, the incidence is lowest after the fiftieth year of age. Tuberculosis has a greater predilection for males in all age groups.

There have been both nontuberculous and tuberculous complications following BCG vaccination. The nontuberculous complications are primary atypical pneumonia, pulmonary infiltration associated with eosinophilia, sarcoidosis, and various diseases of the central nervous system. The tuberculous complications are local, either papulonecrotic or maculopapullar irritative reactions, and lupus has been detected at the vaccination site in ten or twelve cases. The general complication is swelling of the regional lymph nodes with or without secondary infection.

There were four fatal complications in Miettinen's study which were definitely due to BCG vaccination: (*a*) A female infant developed generalized tuberculosis of the lymph nodes with disseminated abscesses, and died at the age of fifteen months. (*b*) A 7-year-old boy died two years after BCG vaccination, similarly with generalized lymphatic tuberculosis. (*c*) A 19-year-old man developed tuberculous meningitis and died

five years after vaccination. (*d*) An 8-month-old boy who was vaccinated two days after birth died of generalized lymphatic tuberculosis.

Miettinen has set out to discover in this monograph:

(*1*) Does eye tuberculosis occur in BCG-vaccinated subjects?

(*2*) If eye tuberculosis occurs, are there differences in the symptoms and course of the disease when compared with those of unvaccinated subjects?

(*3*) Can BCG vaccination affect the nature of a tuberculous eye disease?

Some rigid specifications established by Miettinen had to be satisfied before an eye disease was placed under the general classification of "tuberculous": (*1*) The patient must react positively to a tuberculin test or, if no tuberculin test has been performed, it must be possible to indicate a moderately advanced tuberculous focus by other means, namely, by locating bacilli histologically or roentgenographically; (*2*) it must be possible to eliminate all other etiologic factors; (*3*) the clinical picture of the eye disease must not preclude tuberculosis; (*4*) the patient must respond to tuberculosis therapy.

A total of 149 patients were vaccinated with BCG (15 per cent of the total material). There were 73 cases of proved tuberculous eye disease according to the categories enumerated above. Males comprised 40 per cent of the vaccinated group, and females, 60 per cent. Only 17 per cent of the vaccinations were done in patients more than fifteen years of age. Miettinen's material does not include a single case in which the patient contracted any sort of eye inflammation after a tuberculin test. A total of 18 patients in whom tuberculosis was present were BCG vaccinated. In none of the cases was there evidence that the disease was activated by BCG vaccination performed after the contraction of an infection. This result agrees with Ustvedt's opinion. In 35 cases there was no information concerning the time of vaccination and the subsequent eye disease. There were 32 cases in which the time interval between the BCG vaccination and the subsequent eye disease was known. In no case was there any relationship between the BCG vaccination and the onset of the disease.

It is theoretically possible that BCG vaccination may, in individual cases, provoke symptoms of phlyctenular keratoconjunctivitis fifty-five to sixty-eight days after the vaccination. Several investigators besides Miettinen state that only the cases in which the disease appears three to three and one-half months after vaccination can be classified as primary post-BCG vaccination tuberculosis.

It must be remembered that a tuberculous infection in cases of sclerokeratitis, retinal periphlebitis, iridocyclitis, choroiditis, and uveitis cannot be regarded as having occurred after vaccination unless at least six months have elapsed from the date of vaccination. Accordingly, in cases in which the disease appears in less than six months, infection must be considered to have occurred prior to BCG vaccination.

In summary, it may be stated that tuberculosis of the eye is rare in the BCG-vaccinated patient, and that in all patients in the present study in whom vaccination was performed, the symptoms of the ocular disease emerged only when the protection conferred by the BCG vaccination had disappeared.

The second part of Dr. Miettinen's investigations is a search for a reply to the question "Is there any difference between the BCG-vaccinated and nonvaccinated with regard to the course and nature of the disease?" The small size of the test material makes it difficult to find a clean-cut answer; the general impression gained from the present material, however, is that no noteworthy differences existed between the vaccinated and the nonvaccinated.

The purpose of the third part of this investigation was to decide whether the nature of a tuberculous eye disease is affected by BCG vaccination. It was found that BCG vaccination may cause, in certain tuberculin-negative persons, symptoms similar to those of a tuberculous eye disease, but that it cannot be proved with certainty that BCG vaccination is capable of affecting the nature of a tuberculous eye disease.

In all of the cases in which BCG vaccination was performed correctly, the interval between vaccination and the onset of tuberculosis of the eye was more than five years. It is an established fact that by the time tuberculosis of the eye appears, except for phlyctenular keratoconjunctivitis, tubercle bacilli have been present in the patient's blood for a long time.

The low incidence of tuberculosis of the eye in BCG-vaccinated patients correlates well with findings in this respect in tuberculosis of other organs by other Finnish investigators.

This excellent, well-documented, critical, case-by-case analysis of BCG-vaccinated persons in relation to eye diseases is a distinct and valuable contribution to the ophthalmic literature. There are many excellent tables throughout the monograph which readily explain and summarize the detailed analyses of these cases.

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ANTIBIOTICS MONOGRAPHS No. 12: ANTIBIOTIC THERAPY FOR STAPHYLOCOCCAL DISEASES. Edited by Henry Welch and Maxwell Finland. Pp. 208, Medical Encyclopedia, Inc., New York, 1959. Price, $4.50.

This short manual contains a great deal of factual data. The first chapter deals with penicillin, chloramphenicol, the tetracyclines, and bacitracin; subsequent chapters deal with erythromycin, oleandomycin, novobiocin, vancomycin, ristocetin, and kanamycin. The authors, fortunately, have retained the simple classification of the organism into *Staphylococcus aureus* and *albus*, but the fact that they have used human rather than rabbit plasma for the detection of coagulase production is open to criticism.

Perhaps the best chapters, both factually and for ease of reading, are those by Drs. Maxwell Finland and William M. M. Kirby, which points up a minor defect of the manual, namely, an unevenness in readability and in content. For example, oleandomycin, a useful but less frequently used antimicrobial, receives 58 pages of comment and 152 references, as opposed to 10 pages and 39 references on erythromycin. The chapter on oleandomycin is bedevilled with chemistry and infrared spectra which appear to be inconsistent with the avowed purpose of the book: to guide the physician as to "where, how, when and why to use each one of these drugs." Throughout the book, emphasis is rightly placed on the use of surgical drainage as an adjuvant to chemotherapy.

There is very little evidence of factual error. One wonders what evidence is available that "staphylococcal toxins may play a role in the pathogenesis of this disease" (staphylococcal pneumonia).

This is a useful book containing all the information essential to an understanding of the treatment of staphylococcal infections, which are seen reasonably frequently as complications of the surgical treatment of tuberculosis and as problems in the care of medical and ancillary personnel of sanatoriums. It can be recommended with the above-stated reservation on the unevenness in presentation, which leads to a certain loss in readability. The manual is up-to-date, full of useful information, and pleasantly produced.

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SECHZIG JAHRE MEDIZINISCHE RADIOLOGIE. Probleme und Empirie. By Hans R. Schinz. Pp. 275, Georg Thieme Verlag, Stuttgart (in U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $4.65.

Professor Schinz of Zürich, the author of this delightful little book, must be given great credit for having devoted his time to such a worthy task. His historical review was written for the participants of the IX International Congress for Radiology which took place in Munich in July, 1959. The interested reader will find a wealth of fascinating information on the discovery of the roentgen ray, the efforts of the early pioneers to adapt this newly detected phenomenon to medical use, the development of the diagnostic procedures, therapy with radioisotopes, and the gradual recognition of the potential dangers of radiation. Numerous photographs are included of the men and women who, through their tireless efforts, made the X-ray machine one of the most powerful instruments of modern medicine. It was a particularly excellent idea to reprint in an appendix six fundamental publications by Röntgen, Becquerel, P. and M. Curie, Rutherford, Hahn, and Strassmann, each of which ushered in a new period of human progress. Aside from the importance of their contents, each one of these classics constitutes a first-class piece of clear medical writing, as, for instance, the first communication by Pierre and Marie Curie on the discovery of radium.

In conclusion: This is a highly valuable little book, marvelously suited for fireside reading, even for scholars with only scant knowledge of German.

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American Thoracic Society

Medical Section of the National Tuberculosis Association

PRELIMINARY PROGRAM, MEDICAL SESSIONS, AMERICAN THORACIC SOCIETY ANNUAL MEETING

MONDAY, MAY 22

10:45 a.m.—12:30 p.m.

1A Symposium

Air Pollution—Its Impact on Acute and Subacute Respiratory Diseases—RICHARD A. PRINDLE, M.D., Washington, D.C., *Moderator*
(Participants to be announced)

12:45 p.m.—2:00 p.m.

Sandwich Seminar I*

Differential Diagnosis of Noncardiac Chest Pain—H. WILLIAM HARRIS, M.D., Philadelphia, Pa., *Moderator*
(Panelists to be announced)

Sandwich Seminar II*

Management of Patients with Severe Acute Nontuberculous Lung Disease—ROGER S. MITCHELL, M.D., Denver, Colo., *Moderator*
(Panelists to be announced)

Sandwich Seminar III*

Management of Psychiatric Problems in the Tuberculosis Hospital—EMIL ROTHSTEIN, M.D., Brockton, Mass., *Moderator*
(Panelists to be announced)

2:00 p.m.—4:00 p.m.

2A Diagnostic Techniques—(Chairman to be announced)

Cinebronchography—WILLIAM MOLNAR, M.D., Columbus, Ohio

Abnormalities of the Pulmonary Artery Resembling Bronchogenic Carcinoma—WILLIAM B. BUCKINGHAM, M.D., GEORGE C. SUTTON, M.D., and WILLIAM T. MESZAROS, M.D., Chicago, Ill.

Use of Superheated Saline Aerosols as a New Effective Diagnostic Measure in Pulmonary Tuberculosis—GUSTAV J. BECK, M.D., New York, N.Y.

Accuracy of Examination of Pleural Fluid for Malignant Cells—C. RICHARD GILL, M.D., DAVID T. CARR, M.D., and LEWIS B. WOOLNER, M.D., Rochester, Minn.

Buttonhook-Type Needle Biopsy of Pleura and Rib in Thoracic Disease—HAROLD LEVINE, M.D., PAUL B. SZANTO, M.D., and DAVID W. CUGELL, M.D., Chicago, Ill.

The Importance of Biopsying Non-Palpable Scalene Nodes in Bronchogenic Carcinoma—BERTHA MAZUR, M.D., and GEOFFREY L. BRINKMAN, M.D., Detroit, Mich.

Lung Biopsy via Limited Thoracotomy in Twenty-Four Patients with Undiagnosed

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* Each Seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Thoracic Society.

Alveolar Capillary Block Syndrome—Nola M. Moore, M.D., Helen A. Dickie, M.D., John Rankin, M.D., and Walter H. Jaeschke, M.D., Madison, Wisc.

Pulmonary Vascular Shunts: Measurement and Localization Using the O_2 Electrode and Temporary Unilateral Pulmonary Artery Occlusion—Edward W. Swenson, M.D., David C. Funk, M.D., and Santiago V. Guzman, M.D., San Francisco, Calif.

2:00 p.m.—4:00 p.m.

2B Fungous Diseases

Occurrence of Cryptococcus Neoformans *in the Environment of Three Associated Cases of Cryptococcal Meningitis*—H. G. Muchmore, M.D., E. R. Rhoades, M.D., G. E. Nix, B.S., and R. E. Carpenter, M.D., Oklahoma City, Okla.

Pulmonary Aspergilloma: Pathogenesis, Clinical Significance, and Relationship to Histoplasmic Cavities—Jan Schwarz, M.D., and Gerald L. Baum, M.D., Cincinnati, Ohio

Dissemination of Coccidioidomycosis in Patients with Neoplastic Diseases—Drake W. Will, M.D., John F. Murray, M.D., Sydney M. Finegold, M.D., Vera L. Sutter, Ph.D., and Benjamin J. Fishkin, M.D., Los Angeles, Calif.

Chemotherapeutic Studies on a New Anti-fungal Antibiotic, X-5079C, Effective Against Systemic Mycoses—E. Grunberg, Ph.D., J. Berger, Ph.D., and E. Titsworth, B.S., Nutley, N.J.

Chemotherapeutic Activity, Toxicity, and Serum Assay of a New Antimycotic Antibiotic, X-5079C—Chester W. Emmons, Ph.D., G. W. Lones, Ph.D., and W. R. Piggott, Bethesda, Md.

The Therapeutic and Toxic Activity of X-5079C in Systemic Mycoses of Man—John P. Utz, M.D., Seymour M. Sabesin, M.D., and Vincent T. Andriole, M.D., Bethesda, Md.

Study of the Bacterial Content of Sputum and Saliva in Patients with Chronic Bronchial Disease—Ralph A. Knight, Ph.D., Ruthe A. Okawa, B.S., Salt Lake City, Utah, and H. William Harris, M.D., Philadelphia, Pa.

The Effects of Four Antimicrobial Regimens on the Incidence of Sputum Superinfection in Hospitalized Patients—Donald B. Louria, M.D., New York, N.Y.

2:00 p.m.—4:00 p.m.

2C Metabolism of Mycobacteria—(Chairman to be announced)

Studies on the Arylphosphatase Activity of Mycobacteria—George P. Kubica, Ph.D., Atlanta, Ga.

Intermediate Pathways of Glucose Metabolism by Mycobacterium Smegmatis—Rudolf E. Noble, Ph.D., Dieter Koch-Weser, M.D., and Ernest P. Noble, Ph.D., Cleveland, Ohio

Preliminary Observations on the Relationship Between Glycolipid Composition and Colony Morphology of Different Strains of Mycobacteria—Giancarlo B. Fregnan, D. Biol., Donald W. Smith, Ph.D., and Harrison M. Randall, Ph.D., Madison, Wisc.

Mycoside F, A New Glycolipid Characteristic for Mycobacterium Fortuitum—Harrison M. Randall, Ph.D., S. V. Rao, Ph.D., Giancarlo B. Fregnan, D. Biol., and Donald W. Smith, Ph.D., Madison, Wisc.

Serological Responses with Tuberculo-Immunity and Tuberculosis—Irene G. Melvin, M.S., and H. Mac Vandiviere, M.D., Chapel Hill, N.C.

Alterations of Colony Morphology of Mycobacteria Associated with Lysogeny—Arthur White, M.D., Frances Foster, A.B., and Linda Lyons, B.S., Louisville, Ky.

The Bacteriophage Susceptibility of the Battey Type of Unclassified ("Atypical")

Acid-Fast Bacillus and M. Avium *Isolated from Fowl and Swine: An Epidemiologic Study*—SEYMOUR FROMAN, PH.D., Olive View, Calif.

4:30 p.m.—6:00 p.m.

3A Round Table

New Techniques of Tuberculin Testing—DAVID T. SMITH, M.D., Durham, N.C., *Moderator*

Participants: FLOYD M. FELDMANN, M.D., New York, N.Y., MICHAEL L. FURCOLOW, M.D., Kansas City, Kans., LYDIA EDWARDS, M. D., Washington, D.C., and ROBERT J. ATWELL, M.D., Columbus, Ohio

3B Round Table

Pulmonary Hypertension—WILLIAM W. STEAD, M.D., Milwaukee, Wisc., *Moderator*

Participants: CRAIG W. BORDEN, M.D., Chicago, Ill., J. FRANCIS DAMMANN, JR., M.D., Charlottesville, Va., NOBLE FOWLER, M.D., Cincinnati, Ohio, and JOHN B. HICKAM, M.D., Indianapolis, Ind.

TUESDAY, MAY 23

9:00 a.m.—10:45 a.m.

4A Symposium

Viral Infections and Chronic Bronchopulmonary Disease—EDWIN D. KILBOURNE, M.D., New York, N.Y., *Moderator*

(Participants to be announced)

11:00 a.m.—12:00 Noon

The Amberson Lecture—(Chairman to be announced)

Recent Advances in Pulmonary Physiology—JULIUS H. COMROE, JR., San Francisco, Calif.

2:00 p.m.—4:00 p.m.

5A Emphysema—(Chairman to be announced)

Classification and Prognosis of Diffuse Pulmonary Emphysema: An Analysis of 123 Patients—D. BOYD BIGELOW, LOUIS HAGLER, GILES FILLEY, M.D., and ROGER S. MITCHELL, M.D., Denver, Colo.

The Use of Rapid Simple Pulmonary Function Tests as Screening Devices in the Detection of Chronic Respiratory Disease—CHARLES I. LEFTWICH, M.D., R. MORTON MANSON, M.D., and ROBERT D. ROWAN, M.D., San Jose, Calif.

Chronic Bronchitis and Emphysema: A Study of Day-to-Day Variations in the Disease—WILLIAM S. SPICER, JR., M.D., WILLIAM KEITH MORGAN, M.D., PATRICK B. STOREY, M.D., H. DAVID KERR, M.D., and NATALIE ELIZABETH STANDIFORD, R.N., Baltimore, Md.

The Effects of Intermittent Positive Pressure Breathing (IPPB) on Intrathoracic Pressure, Pulmonary Mechanics, and the Work of Breathing—STEPHEN M. AYRES, M.D., ROBERT KOZAM, M.D., LEWIS L. Y. LI, M.D., and DANIEL S. LUKAS, M.D., New York, N.Y.

A Factual Explanation of the Success of Tracheal Fenestration in the Treatment of Respiratory Insufficiencies—E. E. ROCKEY, M.D., C. F. BLAZSIK, M.D., and S. A. THOMPSON, M.D., New York, N.Y.

Study in the Application of Physical Medicine and Rehabilitation to Emphysema Patients—ALBERT HAAS, M.D., ALEKSANDER LUCZAK, M.D., ROGER KERNISANT, M.D., and VERA ZOTOVIC, M.D., New York, N.Y.

Comparison of Wet and Dry Inflation Techniques for Fixing, Staining, and Mount-

ing Lung Specimens—Daniel E. Jenkins, M.D., David Bahar, M.D., Heinz Loth, B.A., and Hollis G. Boren, M.D., Houston, Texas

Correlation of Postmortem Function and Structures in Normal and Emphysematous Lungs—Philip C. Pratt, M.D., M. Anwarul Haque, M.D., and George A. Klugh, A.B., Columbus, Ohio

2:00 p.m.—4:00 p.m.

5B Therapy—Clinical Tuberculosis—(Chairman to be announced)

The Specific Serum Antimycobacterial Activity Test as a Guide to the Appropriate and Adequate Chemotherapy of Pulmonary Tuberculosis—Riaz Gill, M.D., William F. Russell, Jr., M.D., William E. Dye, Ph.D., and Irving Kass, M.D., Denver, Colo.

High-Dose Isoniazid Compared with Standard-Dose Isoniazid, with PAS, in the Treatment of Previously Untreated Cavitary Pulmonary Tuberculosis—H. William Harris, M.D., Philadelphia, Pa.

Isoniazid Alone Compared with Isoniazid and PAS in the Treatment of Previously Untreated Noncavitary Minimal and Moderately Advanced Pulmonary Tuberculosis—W. Spencer Schwartz, M.D., Oteen, N.C.

Methyl Prednisolone in the Treatment of Pulmonary Tuberculosis—J. R. Johnson, M.D., B. C. Taylor, M.D., J. F. Morrissey, M.D., Madison, Wisc., J. W. Jenne, M.D., G. L. Loeb, M. D., and F. M. MacDonald, M.D., Minneapolis, Minn.

A Clinical Study of Steroid Therapy of Pulmonary Tuberculosis—Nicholas D. D'Esopo, M.D., West Haven, Conn.

Follow-Up on Patients Discharged with Open-Negative Cavity Syndrome—Raymond F. Corpe, M.D., and Frank A. Blalock, M.D., Rome, Ga.

Unclassified (Atypical) Acid-fast Mycobacteria in Childhood Disease (including Tuberculosis)—Gladys Boyd, M.D., T. E. Roy, M.D., and Grace Craig, R.T., Toronto, Canada

Photochromogen Infection with Negative Chest X-Ray—W. B. Buckingham, M.D., and M. Silverman, Ph.D., Chicago, Ill.

2:00 p.m.—4:00 p.m.

5C Laboratory Research—(Chairman to be announced)

A Simple Test to Determine Whether Patients Are Taking Prescribed Isoniazid—John E. Kasik, M.D., and Alfred Heller, M.D., Chicago, Ill.

Tuberculosis in Man, Dog, and Cat—V. M. Hawthorne, M.B., and I. Lauder, M.R.C.V.S., Glasgow, Scotland

Host-Parasite Relationships in Natively Resistant and Susceptible Rabbits on Quantitative Inhalation of Human and Bovine Tubercle Bacilli—Marvin J. Allison, Ph.D., Peter Zappasodi, B.S., and Max B. Lurie, M.D., Philadelphia, Pa.

The Capacities of Various Vaccines to Prevent Induction of Pulmonary Tuberculosis in the Rhesus Monkey—Robert C. Good, Ph.D., Richard A. Hoffman, B.A., Paul N. Jolly, M.D., and L. H. Schmidt, Ph.D., Cincinnati, Ohio

Resistance to Experimental Tuberculosis Following the Injection of Nonviable Preparations of Histoplasma Capsulatum *in the Mycelial Phase*—Loyd W. Hedgecock, Ph.D., Kansas City, Kans.

Specific and Non-Specific Biological Activities of the Tubercle Bacillus—David W. Weiss, Ph.D., D. Phil., and Rose S. Bonhag, M.S., Berkeley, Calif.

Tubercle Formation after Injection of Pine Pollen into Spleens of Rats—A. Lindner, M.D., R. Naik, M.D., T. Kutkam, M.D., J. Sokatch, Ph.D., and J. F. Hammarsten, M.D., Oklahoma City, Okla.

4:30 p.m.—6:00 p.m.

6A Round Table

Adequacy of Outpatient Chemotherapy—THEODORE L. BADGER, M.D., Boston, Mass., *Moderator*

Participants: AARON D. CHAVES, M.D., New York, N.Y., H. D. IRELAND, M.D., Grand Rapids, Mich., and KARL H. PFUETZE, M.D., Chicago, Ill.

6B Round Table

Fractions of the Tubercle Bacillus as Immunizing Agents—GLADYS L. HOBBY, PH.D., East Orange, N.J., *Moderator*

Participants: GUY P. YOUMANS, M.D., Chicago, Ill., DAVID W. WEISS, PH.D., Berkeley, Calif., and ALFRED J. CROWLE, PH.D., Denver, Colo.

6C Round Table

Criteria of Operability for Carcinoma of the Lung—SOL KATZ, M.D., Washington, D.C., *Moderator*

Participants: ROBERT R. SHAW, M.D., Dallas, Texas, RICHARD H. OVERHOLT, M.D., Boston, Mass., OSCAR AUERBACH, M.D., East Orange, N.J., and BENJAMIN FELSON, M.D., Cincinnati, Ohio.

WEDNESDAY, MAY 24

9:00 a.m.—10:30 a.m.

7A Symposium

The Present Management of the Patient with Drug-Resistant Bacilli—JAMES W. RALEIGH, M.D., New York, N.Y., *Moderator*

A Perspective on Drug Resistance—GLADYS L. HOBBY, PH.D., East Orange, N.J.

Chemotherapy of Tuberculosis with Drug-resistant Infections—JAMES H. MATTHEWS, M.D., Washington, D.C.

Drug Resistance in Ambulatory Antituberculous Chemotherapy—AARON D. CHAVES, M.D., ARTHUR B. ROBINS, M.D., HANS ABELES, M.D., and GERTRUDE DANGLER, New York, N.Y.

Pulmonary Resections in Patients Harboring Drug-resistant Tubercle Bacilli—DAVID V. PECORA, M.D., Ray Brook, N.Y.

Long-Term Results of Medical and Surgical Treatment of Pulmonary Disease Due to M. Kansasii—DAVID BAHAR, M.D., IRVING CHOFNAS, M.D., and DANIEL E. JENKINS, M.D., Houston, Texas

10:45 a.m.—12:30 p.m.

8A Tuberculin Testing—(Chairman to be announced)

Experience with the Tuberculin Tine Test in a Sanatorium—D. C. CAPOBRES, M.D., FRED E. TOSH, M.D., J. LEWIS YATES, M.D., and H. V. LANGELUTTIG, M.D., Mt. Vernon, Mo.

The Disc Tuberculin Test (Dried Tuberculin—Disposable Unit)—SOL ROY ROSENTHAL, M.D., Chicago, Ill.

Comparison of Intradermal PPD-S, PPD-B, and a New Intradermal Dried Tuberculin Test—EDITH M. LINCOLN, M.D., and ELISA V. SADILI, M.D., New York, N.Y.

A Comparison of the Intradermal Intermediate PPD Reaction with the Tuberculin Tine Test in 5,066 Adults and Children—HAROLD J. LYNCH, JR., M.D., FRED E. TOSH, M.D., IRENE L. DOTO, M.A., VIRGIL A. PLACE, M.D., and MICHAEL L. FURCOLOW, M.D., Kansas City, Kans.

Heaf Test for Survey—KATHARINE H. K. HSU, M.D., Houston, Texas

Results of County-wide Mantoux Testing among School Children in the Middle West in 1960—Michael L. Furcolow, M.D., Irene L. Doto, M.A., Harold J. Lynch, Jr., M.D., Fred E. Tosh, M.D., and Jack C. Evans, M.D., Kansas City, Kans.

10:45 a.m.—12:30 p.m.

8B Pathology—Surgery—(Chairman to be announced)

Multicentric Origin of Carcinoma in Association with Tuberculous Foci—Harry L. Katz, M.D., Brooklyn, N.Y.

Pulmonary Adenocarcinomas and Their Relation to Scars—Raymond A. Yesner, M.D., West Haven, Conn.

Eventration of the Diaphragm—Elliott Michelson, M.D., Baltimore, Md.

Use of the Soviet Bronchus Stapler in Pulmonary Resections—Mark M. Ravitch, M.D., and Felicien M. Steichen, M.D., Baltimore, Md.

A New Technique for the Closure of the Bronchus—Mark W. Wolcott, M.D., Harold Medlin, M.D., and Curtis G. Wherry, M.D., Coral Gables, Fla.

The Resectional Therapy of Pulmonary Tuberculosis in Children—J. L. Wofford, M.D., Watts R. Webb, M.D., and Hans K. Stauss, M.D., Jackson, Miss.

12:45 p.m.—2:00 p.m.

Sandwich Seminar IV*

Treatment of Oligopnoea—Edward Gaensler, M.D., Boston, Mass., *Moderator*

(Panelists to be announced)

Sandwich Seminar V*

Optimum Duration of Chemotherapy—Nicholas D. D'Esopo, M.D., West Haven, Conn., *Moderator*

(Panelists to be announced)

Sandwich Seminar VI*

Changing Status of Administrative Problems in the Tuberculosis Hospital—Harold G. Curtis, M.D., Cleveland, Ohio, *Moderator*

(Panelists to be announced)

2:00 p.m.—4:00 p.m.

9A Pulmonary Physiology—(Chairman to be announced)

The Diffusing Capacity of The Lung in Acute Pulmonary Tuberculosis—M. Henry Williams, Jr., M.D., Nathan S. Seriff, M.D., Turkan Akyol, M.D., and Ok Hi Yoo, M.D., New York, N.Y.

Diffusion Capacity in "Healed" Miliary Tuberculosis—Asher E. Marks, M.D., Loretta Morganti, M.D., and José Bocles, M.D., Miami, Fla.

Determinants of Pulmonary Diffusion in the Experimental Animal—Albert H. Niden, M.D., Benjamin Burrows, M.D., Charles Mittman, M.D., and William R. Barclay, M.D., Chicago, Ill.

Clinical Usefulness of the Single-Breath Diffusing Capacity Test—William R. Barclay, M.D., John E. Kasik, M.D., and Benjamin Burrows, M.D., Chicago, Ill.

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* Each Seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Thoracic Society.

The Oxygen Cost of Breathing at Rest and During Exercise—Louis Hagler, D. Boyd Bigelow, and Giles F. Filley, M.D., Denver, Colo.

Pulmonary Pressure-Flow-Volume Relationships during Maximal Expiratory and Inspiratory Effort—John T. Hagenbucher, M.D., Lawrence Perlman, B.A., Theodore C. Smith, M.D., and John Rankin, M.D., Madison, Wisc.

The Expiratory Pneumotachygram and Intrapulmonary Distribution of Air in Pre-School Children—Gordon L. Snider, M.D., Luis Rivera, M.D., and D. B. Radner, M.D., Chicago, Ill.

Comparison of Measurement of Airway Resistance and Some Common Tests of Airway Obstruction in Normals and Patients with Chronic Pulmonary Diseases—Frank W. Lovejoy, Jr., M.D., and Herbert Constantine, M.D., Rochester, N.Y.

2:00 p.m.—4:00 p.m.

9B Epidemiology—Immunology—(Chairman to be announced)

Skin Reactivity of Selected Indian Groups to Human, Avian, and Battey Tuberculins—Everett R. Rhoades, M.D., George Lythcott, M.D., and Martin M. Cummings, M.D., Oklahoma City, Okla.

Tuberculin Response to Antigens of Contacts of Patients Who Excrete Unclassified (Anonymous) Mycobacteria—John S. Chapman, M.D., and Hal J. Dewlett, M.D., Dallas, Texas

The Cultural Isolation of Mycobacteria from Resected Lung Specimens—Rosemary Colton, B.A., Geoffrey Kent, M.D., and William Lester, M.D., Hinsdale, Ill.

The Isolation of Unclassified (Atypical) Acid-Fast Bacilli from Soil and Water Samples Collected in the State of Georgia—George P. Kubica, Ph.D., R. Edward Beam, B.S., Joseph W. Palmer, and A. Louise Rigdon, B.A., Atlanta, Ga.

The Prevalence of Pneumoconiosis in Coal Miners without Pulmonary Complaints—William H. Anderson, M.D., and Ethem Erinc, M.D., Harlan, Ky.

Separation of the Antigens of Various Mycobacteria—W. T. Kniker, M.D., Little Rock, Ark.

More Specific Response to Some Highly Purified Tuberculoproteins—Javad Vakilzadeh, D.V.M., and H. Mac Vandiviere, M.D., Chapel Hill, N.C.

Allergic Response to Purified Tuberculoproteins in Humans—H. Mac Vandiviere, M.D., Margaret R. Vandiviere, M.A., and Stuart Willis, M.D., Chapel Hill, N.C.

4:00 p.m.—5:30 p.m.

10A Panel

PHS Study on INH Prophylaxis—Edward T. Blomquist, M.D., Washington, D.C., *Moderator*

(*See* General Sessions, Wednesday, May 24.)

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–25
1962—Miami Beach, Florida—May 20–24
1963—Denver, Colorado—May 12–16
1964—New York, New York—May 24–28

8TH ARGENTINE CONGRESS OF PHTHISIOLOGY AND PNEUMONOLOGY

The 8th Argentine Congress of Phthisiology and Pneumonology will be held from November 20–24, 1961, in Bahía Blanca, Argentina. The following subjects are scheduled for presentation: actual epidemiology, the problem of the tuberculous patient with bacilli resistant to two drugs (isoniazid included), surgical treatment of the patient with severe tuberculous disease, chronic pulmonary emphysema, and professional rehabilitation of the tuberculous patient.

Further information may be obtained by writing to the General Secretary, Dr. Jorge A. Pilheu, Juncal 3715, Buenos Aires, Argentina.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant: TERRI MANN

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 42 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk(*) in each abstract title.)

ABSTRACTERS

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Alveolar Hypoventilation and Cardiopulmonary Failure in Obesity. R. Scalettar*, F. L. Miller, D. B. Sodee, and K. Barry. *US Armed Forces Med J*, July, 1960, 11: 774–780. (*Walter Reed Gen. Hosp., Washington, D. C.)

Two cases of the syndrome of alveolar hypoventilation, cardiopulmonary failure, and obesity are presented. The first case demonstrates the borderline state of ventilatory compensation characteristic of these patients, an acute bout of pneumonia resulting in severe cardiac and pulmonary decompensation. Both patients exhibited improvement in clinical manifestations following weight reduction (after authors' summary).—C. H. Pierce

Anthracosilicosis. Selected Aspects Related to the Evaluation of Disability, Cavitation, and the Unusual X-Ray. R. T. Cathcart*, P. A. Theodos, and W. Fraimow. *Arch Intern Med*, September, 1960, 106: 368–377. (*Jefferson Med. College Hosp., Philadelphia, Penn.)

A study was made of 1,690 miners who had worked an average of 20 to 30 years in anthracite fields; almost all had roentgenographic evidence of anthracosilicosis. Frequent discrepancy exists between the degree of clinical disability and the roentgenographic stage of anthracosilicosis. The degree of disability present is related far more closely to the degree of emphysema present than it is to the stage of silicosis. Pulmonary function studies, therefore, are far better correlated with disability than is the chest roentgenogram. Cavitary disease without tuberculosis does occur in anthracosilicosis, and its existence should be recognized. In 33 (18 per cent) of the 182 miners with cavities visible on chest roentgenograms (or 2 per cent of the entire group of miners examined), cavitation was probably related to ischemic necrosis. Clinical features of the ischemic necrosis that may occur in anthracosilicosis are several: (*1*) unilateral or bilateral cavitation in areas of conglomerate fibrosis; (*2*) sudden and rapid development of cavity due to liquefaction of a conglomerate area; (*3*) production of copious amounts of black sputum; (*4*) aseptic course, unaccompanied by chills, fever, or leukocytosis; and (*5*) lack of prognostic influence upon the course of the underlying anthracosilicosis. In 69 per cent of the miners with cavitation, the presence of tuberculosis was readily demonstrated. Lung biopsy has proved helpful both in the evaluation of the equivocal roentgenogram and in the study of the pathogenesis of the disease.
—W. J. Steininger

Argentaffinoma of the Lung with Carcinoid Syndrome. M. Joseph* and R. R. Taylor. *Brit Med J*, August 20, 1960, No. 5198: 568–571. (*Royal Prince Alfred Hosp., Sydney, Australia)

A case is described of argentaffinoma of the lung proved by autopsy examination. The diagnosis was suspected clinically prior to the death of the patient. Only 6 similar cases have been reported in the literature.—E. A. Riley

Asbestosis and Carcinoma of the Lung. J. Anderson* and F. Campagna. *Arch Environ Health*, July, 1960, 1: 27–32. (*St. Mary's Hosp., San Francisco, Calif.)

A case of asbestosis associated with carcinoma of the lung is presented. The literature is reviewed, and this is the 24th reported case, with complete autopsy findings. In general, this case conforms to the previously described cases in which a relationship between the 2 diseases is intimated. It is suggested that further cases of asbestosis, with and without neoplasia, be reported with careful attention to other factors, such as smoking. Only when these facts are available will final assessment of the relationship between asbestosis and lung carcinoma be possible.—T. H. Noehren

Pulmonary Aspergilloma in the Light of an Interesting Case (in Finnish). K. S. Oittinen*. *Duodecim*, August, 1960, 76: 700–706. (*Kirurgi, Helsinki, Finland)

An aspergilloma was removed from the posterior segment of the left upper lobe of a 35-year-old female. Recurrent attacks of hemoptysis over an 8-year-period no longer occurred after the operation. Most probably, the Aspergillus infection was the result of working for long periods of time with reindeer hides.—J. Haapanen

The Status of the Adrenal Gland in the Asthmatic Patient. I. Kass* and S. Appleby. *Amer J Med Sci*, August, 1960, 240: 213-218. (*National Jewish Hosp., Denver, Colo.)

Since 1955, 52 asthmatic adults and 19 control patients with inactive pulmonary tuberculosis were studied to determine the capacity of their adrenal glands to respond to corticotropin. By use of the *t* test for comparing unpaired data, no statistically valid difference was found between the controls and the asthmatic individuals. This statement applied equally well to all groups regardless of whether they had received corticosteroids up to the time of the experiment, had taken none for at least 2 weeks, or were experiencing their first treatment with these hormones. In view of these results, it is probable that adrenal cortical insufficiency was not a factor in the development of bronchial asthma in the patient population studied.
—W. J. Steininger

Hypnotic Treatment of Asthma. G. EDWARDS*. *Brit Med J*, August 13, 1960, No. 5197: 492–497. (*Hammersmith Hosp., England)

Six patients admitted to hospital with severe asthma were treated by hypnotic suggestion. Five were subsequently followed up for not less than 1 year. Patients were assessed both subjectively by their testament, and objectively by spirometry: it was found that an adequate assessment could be based only on a combination of these methods. With this double assessment it was apparent that hypnosis benefited a patient in 1 of 2 entirely different ways: by effecting physiologic improvement (decrease of airways resistance), or by producing psychologic improvement (decreased awareness of airways resistance). The distinction between these 2 responses has not often been adequately stressed. The implications of these findings for psychosomatic theory are discussed. While in hospital, 1 patient completely failed to respond to hypnosis, and 1 responded poorly. Four had complete remissions subjectively, but in only 2 of these was remission complete objectively. Immediate response to hypnosis (before and after sessions) was usually poor, but this could be explained by the content of the suggestion. Speed of remission could be as fast with hypnosis as with physical methods. Three patients relapsed within days of going home, but 2 of these again went quickly into remission. Of the 4 patients originally responding well, 2 thought that their conditions during the following year were much better than they had been previously. Two of these 4 patients were readmitted due to asthma. These results cannot be interpreted as valid evidence for or against the value of hypnosis: an uncontrolled series of 6 patients can be regarded only as a pilot study. The results do, however, strongly suggest that a controlled clinical trial of hypnosis would repay the effort. The particular value of hypnosis may be as an alternative to steroid treatment (after author's summary). —E. A. RILEY

A Syndrome of Ataxia, Oculocutaneous Telangiectasia, and Sinopulmonary Infections. B. F. ANDREWS*, F. M. KOPACK, and O. C. BRUTON. *US Armed Forces Med J*, May, 1960, 11: 587–592. (*Walter Reed Army Hosp., Washington, D. C.)

A detailed clinical history is presented of a 10-year-old white boy with the syndrome of ataxia, oculocutaneous telangiectasia, and sinopulmonary infections. This brings the number of cases reported in the literature to 21. The apparent hypogammaglobulinemia in the patient described here suggests that supplementary gamma globulin may be of possible value in the control of recurrent infections.—C. H. PIERCE

Chronic Bronchitis and Pulmonary Emphysema (in Japanese). T. NAKAMURA*, T. TAKIZAWA, and T. TAKASHIMA. *Saishin Igaku*, August, 1960, 15: 2024-2034. (*Tohoku U., Sendai, Japan)

Chronic bronchitis has an important role in the development of chronic pulmonary emphysema. Bronchiolitis and alveolar changes develop during the course of chronic bronchitis, especially during the phase of acute exacerbation. Decreased elasticity of the lung due to changes in the alveolar structure is an essential factor in the development of pulmonary emphysema. Obstructive disturbances due to asthma, crises of coughs, and hyperventilation accelerate hyperinflation of the lung, and air trapping due to these factors further helps the progress of emphysema. On the other hand, senile changes of the lung result in enlargement of respiratory bronchioles and alveolar tracts and, finally, destruction of alveoli compatible with emphysematous changes. Although repeated infection and other environmental factors will help these changes develop into chronic pulmonary emphysema, some forms of chronic emphysema may be the result of progressive senile and degenerative changes of the lungs without infection.—I. TATENO

Induced Sputum as a Diagnostic Technique for Cancer of the Lungs. A. L. BARACH*, H. A. BICKERMAN, G. J. BECK, K. G. S. NANDA, and E. R. PONS, JR. *Arch Intern Med*, August, 1960, 106: 230–236. (*College of Physicians and Surgeons, Columbia U., N.Y.)

An improved technique of sputum induction is described for the cytologic diagnosis of lung cancer and other conditions. A new apparatus delivers inhalation aerosols with a temperature of between 115° and 125°F., which appears at present to be optimal for the deposition of water vapor and aerosol droplets on the mucosa of the tracheobronchial tree. In a preliminary study of 14 patients with suspected lung cancer, the presence of malignant cells was demonstrated by the aerosol technique in some instances when routine sputum analysis was negative. Of the 6 spontaneous-cough specimens reported as negative for malignant cells, 3 were diagnosed as positive and 3 as suspicious for abnormal cells when sputum was obtained following the inhalation of the heated aerosol. The simplicity of this technique, the lack of adverse reactions, and its wide acceptance by the subjects tested make it a valuable tool for cytologic and bacteriologic diagnoses of a variety of lung disorders.—W. J. STEININGER

Cancer Development in Pulmonary Scars (in German). R. POHL*. *Fortschr Roentgenstr Nuklearmed*. March 1960. 92: 267–278. (*Barm-

herzige Brüder Spital, Vienna, Austria)

The concept of "scar cancer" is discussed and 5 cases are reported in detail. The primary tumor usually remains small, is peripherally located, and shows an early tendency for hematogenous dissemination. The shape of the primary tumor is irregular, linear to plate-like, but not spherical. —H. ABELES

Cannabosis (in Spanish). A. BARBERO*. *Rev Esp Tuberc*, June, 1960, 29: 285–296. (*Dispensario Central, Alicante, Spain)

This disorder is a pneumoconiosis that may develop in hemp-processing workers. There is an initial reversible phase with acute symptoms and, if the worker does not leave this type of work, it may progress to a secondary phase in which the disease becomes chronic with irreversible features such as fibrosis, bronchitis, emphysema, bronchial asthma and, eventually, cor pulmonale. The importance of prevention is emphasized.—Z. VIRÁGH

Pulmonary "Alveolar Cell" (Bronchiolar) Carcinoma: Report of Three Cases in Thailand. S. BOVORNKITTI*, P. KANGSADAL, S. KARNCHANAKUNCHORN, and S. TANDHANAND. *Tubercle*, August, 1960, 41: 268–271. (*Siriraj Hosp. Med. School, Dhonburi, Thailand)

Three cases of pulmonary "alveolar cell" carcinoma are reported for the first time in Thailand. All had diffuse opacities in the lung fields with pleural effusion, the fluid containing malignant cells. All died from suffocations and the diagnoses were confirmed by postmortem examinations (Authors' summary).—M. J. SMALL

The Differential Diagnosis of Pulmonary Cavities: Pulmonary Periarteritis Nodosa (in German). G. RITTER*. *Tuberkulosearzt*, June, 1960, 14: 346–350. (*Deceased)

Two cavitary lesions were revealed in a 41-year-old male. The first diagnosis was pulmonary tuberculosis; this was later changed to abscesses. Drainage and topical treatment of the anterior cavity proved ineffective. Bacteriologic examination of the aspirated fluid failed to bring out any microorganisms. The development of paresthesia of one leg, bilateral pleural effusions, rise of blood pressure, and the appearance of granular casts in the urinary sediment established the diagnosis of periarteritis nodosa. The autopsy revealed severe obliterative lesions of the pulmonary arteries, focal hemorrhagic consolidation and areas of infarction and necrosis in both lungs, serofibrinous pleurisy, slight obliterative arteritis of the hilar lymph nodes and spleen, severe glomerulitis of both kidneys, obliterative lesions of the cortical arterioles, and tubular nephrosis.—J. HAAPANEN

Chest Diseases in Biblical Times. E. R. SMITH*. *Brit J Dis Chest*, July, 1960, 54: 226–233. (*Stockport Chest Clinic, England)

This paper reports a long journey tracing the knowledge of chest diseases from the Ganges Valley through Assyria and Babylon to Palestine and Egypt, and back again through Palestine to Greece and Rome. How each country and race in turn was influenced by chest disease—with the exception of Israel—is discussed. There is no reference in the Bible, or in fact in any other literature of that period, to any case of tuberculosis among the Hebrews—and, what is still more significant, there was no word for cough in the ancient Hebrew language.—M. J. SMALL

Primary Mediastinal Echinococcosis. J. RAKOWER* and H. MILWIDSKY. *Amer J Med*, July, 1960, 29: 73–83. (*Rothschild Hadassah U. Hosp., Jerusalem, Israel)

The clinical and pathologic features of mediastinal echinococcosis are discussed in the light of 6 cases presented and a review of 74 cases reported in the literature. Mediastinal hydatid cysts are usually multivesicular, except for those in the middle mediastinum. Fifty-five per cent of all of the cases of mediastinal hydatid cysts were located in the posterior mediastinum. Erosion of the ribs and vertebrae and compression of the spinal cord occurred in 57 per cent of all posteromediastinal hydatid cysts. In 36 per cent of the cases the mediastinal echinococcosis was located in the anterior mediastinum; compression of the trachea with severe dyspnea or strangulation was the major complication. In echinococcosis of the middle mediastinum (9 per cent of all cases), compression and erosion of the great vessels is the main danger. Surgery is the treatment of choice and the only means of establishing the final diagnosis.—T. H. NOEHREN

Arteriovenous Fistula of the Coronary Artery (in German). W. PORSTMANN* and W. GEISSLER. *Fortschr Roentgenstr Nuklearmed*, August, 1960, 93: 143–150. (*Charité, Berlin, Germany)

A congenital arteriovenous fistula between the right coronary artery and the veins of the right heart was demonstrated *in vivo* in a 45-year-old man. The patient had a loud systolic-diastolic murmur along the left sternal border. The findings of the electrocardiographic and phonocardiographic examinations and of the right and left heart catheterizations are reported in detail. —H. ABELES

Pulmonary Emphysema: Report of 26 Cases Followed to Death. D. CARROLL*. *Bull Johns Hopkins Hosp*, No. 3, 1960, 106: 154–177. (*Car-

diopulmonary Lab., Baltimore City Hosp., Md.)

A report is made of 26 patients hospitalized for pulmonary emphysema who had serial pulmonary function studies and who died. The inclusion of patients who died ensures that the group was made up of the most severe cases of pulmonary emphysema. Although dyspnea, orthopnea, right heart failure, and hypercapnia are relatively poor prognostic signs and symptoms, death appears to occur at times of acute bronchial infection and uncontrolled heart failure. As long as infection and heart failure can be prevented, the patient survives. Once these complications supervene, vigorous treatment may allow the patient to survive for prolonged periods but, sooner or later, a time comes when response to vigorous treatment fails. Serial physiologic studies demonstrated moderate variations in function studies when the clinical status appeared unchanged. Such variations make an evaluation of therapeutic measures very difficult, except in acute respiratory acidosis. Three patients who had repeated control studies were rendered myxedematous with radioactive iodine. In 2, there was no clinical or physiologic measurement suggestive of improvement. In the third (who still survives) there was definite subjective improvement, but no change in the physiologic studies (after author's summary).—C. H. Pierce

Fatal Gastro-intestinal Hemorrhage in Hodgkin's Disease. Nine Cases. S. B. Anderson*. *Acta Med Scand*, July 1, 1960, 167: 193–197. (*Bispebjerg Hosp., Copenhagen, Denmark)

The present paper adds 9 further cases to the 27 cases of fatal gastrointestinal hemorrhage in lymphogranulomatosis already recorded in the literature. In 3 of the 9 cases, bleeding came from lymphogranulomatous ulcerations. Three of the remaining patients had ordinary duodenal ulceration. In the other 3 cases, the source of hemorrhage was unknown. For 2 reasons, fatal gastrointestinal hemorrhage in lymphogranulomatosis must be anticipated to increase in frequency. The average life expectancy of these patients has become longer—and the longer symptoms have been present, the more frequently has the gastrointestinal tract been involved. Treatment with steroids is at present employed to a very great extent, and may probably activate and precipitate hemorrhage from ordinary peptic ulcers.—E. Dunner

Gynecomastia Associated with Lung Cancer. J. Hardy*. *JAMA*, July 30, 1960, 173: 1462–1465. (*U. of Mississippi, Jackson)

Three cases of gynecomastia occurred in association with bronchogenic carcinoma. Some degree of testicular atrophy was observed in all 3 patients. —H. Abeles

Mediastinal Cystic Hygroma: Report of Two Cases. M. B. Divertie*, R. A. Lim, E. G. Harrison, P. E. Bernatz, and T. C. Burger. *Proc Mayo Clin*, August 3, 1960, 35: 460–467. (*Mayo Clinic, Rochester, Minn.)

Two cases of mediastinal cystic hygroma are reported and the clinical, pathologic, and developmental features are discussed. This type of tumor cannot be distinguished from other mediastinal tumors without thoracotomy.—E. A. Riley

Familial Hypogammaglobulinemia, Splenomegaly and Leukopenia. D. Kushner*, A. Dubin, W. Donlon, and D. Bronsky. *Amer J Med*, July, 1960, 29: 33–42. (*U. of Illinois, Chicago)

The death from disseminated histoplasmosis of a patient with hypogammaglobulinemia, increased susceptibility to infection, splenomegaly, and leukopenia led to an investigation of his family. Three of his 6 living children, a 13-year-old boy and 6-year-old fraternal twin girls, were found to have a similar syndrome without evidence of infection with histoplasmosis. The patient's father had splenomegaly, but no other features of this syndrome. The other members of the family had none of these abnormalities. In the involved members the level of gamma globulins and the degree of immunologic responsiveness, while depressed, were greater than usually observed in other forms of hypogammaglobulinemia. The evidence suggests that this apparently new clinical entity is an hereditary disturbance transmitted possibly by an autosomal dominant gene (Authors' summary). —T. H. Noehren

The Familial Occurrence of Hypoplastic Right Lung with Systemic Arterial Supply and Venous Drainage "Scimitar" Syndrome. C. A. Neill*, C. Ferencz, D. C. Sabiston, and H. Sheldon. *Bull Johns Hopkins Hosp*, No. 1, 1960, 107: 1–15. (*Johns Hopkins U. School of Med., Baltimore, Md.)

A father and daughter are reported who had identical anatomic malformations of the right lung: the right lung was smaller than normal, the right pulmonary venous return was into the inferior vena cava, and there was systemic arterial supply to the right lower lobe. The father had a mild degree of dextroposition of the heart and pursued an asymptomatic course without pulmonary hypertension. The daughter had a gross degree of dextroposition of the heart with marked hypoplasia of the right lung and right pulmonary artery: she had frequent respiratory infections, severe pulmonary hypertension, and died following right pneumonectomy. A review of similar cases in the

literature revealed that this malformation is usually benign, as it was in the father, but symptoms are rarely present from an early age. The clinical course seems more severe in those with marked hypoplasia of the right lung. Severe pulmonary hypertension, as observed in the child, has not been previously reported. Microscopic studies revealed pulmonary hypertensive lesions of unusual severity in the small arteries of the left, otherwise normal, lung. These changes were not seen in those parts of the right lung supplied by the right pulmonary artery. The data available suggest the pulmonary hypertension was of vasomotor origin, related either to the extreme degree of hypoplasia of the right lung and its artery or, possibly, to obstruction of the anomalous pulmonary vein. Surgery for this malformation appears to be of limited value (after authors' summary).
—C. H. Pierce

Pulmonary Complications During Mecamylamine Therapy. R. Rokseth* and O. Storstein. *Acta Med Scand,* May 20, 1960, 167: 23–27. (*Nordland County Hosp., Bodö, Norway)

Severe pulmonary changes occurring during treatment with mecamylamine are described in a patient with malignant hypertension. In addition to the fibrinous masses in the lung alveoli and the fibrous thickening of the alveolar walls described earlier after use of hexamethonium and pentolinium, extensive pathologic changes of the blood vessels were found.—E. Dunner

Microlithiasis Alveolaris Pulmonum. M. H. Waters*. *Tubercle,* August, 1960, 41: 276–278. (*Spynie Hosp., Elgin, Scotland)

This paper describes 2 further cases of this rare disease which occurred in sisters. One interesting finding roentgenographically (apparently so far not reported) was observed in the 2 cases described, both of which showed at some stage small pneumothoraces at the apices. No cause for the formation of the pulmonary concretions has been proved so far, and the disease remains a medical mystery.—M. J. Small

Monosporium Mycetoma of the Lung (in German). H. Stoeckel* and Ch. Ermer. *Beitr Klin Tuberk,* March, 1960, 122: 30–38. (*Tuberkulose-Forschungsinstitut, Berlin-Buch, Germany)

A 53-year-old gardener underwent pulmonary resection for a preoperative diagnosis of aspergilloma. From the resected specimen, *Monosporium apiospermum* (Saccardo 1911) was cultured. Animal inoculation proved the fungus to be pathogenic.—Z. Virágh

Data on the Problem of Pleurisy (in Hungarian). A. Papp*, J. Stark, M. Katona, and M. Foki. *Tuberkulozis,* February, 1960, 13: 50–53. (*Fodor József Tbc. Gyógyintézet, Budapest, Hungary)

Pleural effusion in the adult is of tuberculous origin in 82 to 92 per cent of the cases found in the literature. Pulmonary or extrapulmonary tuberculosis may develop within 5 years after the pleurisy. This occurred in 15 (10 per cent) of 150 patients treated with antimicrobials on an outpatient basis. Of 41 patients who received antimicrobial treatment combined with corticotropin in hospital, only 1 was found to have recidivation (erythema nodosum) in a 1- to 6-year follow-up study. The importance of hospitalization of patients with pleural effusion is emphasized.—Z. Virágh

Loeffler's Pneumonia Due to Oxyuriasis (in Spanish). M. Cabal Gonzalez*, A. Suarez Estrada, and J. Fernandez Cuervo. *Rev Esp Tuberc,* June, 1960, 29: 265–274. (*Dispensario Comarcal Antituberculoso De Aviles, Oviedo, Spain)

The case history is presented of a 31-year-old man diagnosed as having eosinophilic infiltration who was successfully treated with antiparasitic and antihistamine drugs.—Z. Virágh

Pulmonary Alveolar Proteinosis. W. Edmondson* and J. Gere. *Ann Intern Med,* June, 1960, 52: 1310–1318. (Orange Mem. Hosp., Orange, N. J.)

The clinical, laboratory, and biopsy findings in a 53-year-old man with pulmonary alveolar proteinosis are presented. From observations on this isolated case the authors are unable to make any suggestions as to the etiology of this disease. This is the third reported occurrence of the disease in an American Negro.—T. H. Noehren

Course and Prognosis of Sarcoidosis. M. Sones* and H. Israel. *Amer J Med,* July, 1960, 29: 84–93. (*Jefferson Med. College, Philadelphia, Pa.)

Observations are presented on the course of sarcoidosis in 211 patients (184 Negroes and 27 Caucasians) observed in Philadelphia for as long as 20 years, with a mean interval of 5.9 years. Survival rates, calculated by the life-table method, were 88.8 per cent after 5 years of observation and 84.8 per cent after 10 years. Comparison with the life expectancy of normal persons similar in age, sex, and race indicates a considerable diminution of survival as the result of sarcoidosis. Complete recovery was observed in 34.6 per cent and improvement in 31.8 per cent, while in 16.1 per cent the condition became worse. The mortality rate was 9.5 per cent, with 16 deaths due to sarcoidosis, 3 to tuberculosis, and 1 to incidental disease.

Tuberculosis developed in 4 other patients, 1 of whom was among the 93 patients treated with corticosteroids. In only 2 instances was recurrence of sarcoidosis observed. The incidence of erythema nodosum was much less frequent than reported in English and Scandinavian studies, and clearing of mediastinal adenopathy occurred less often and less rapidly than in these 2 countries. With these exceptions, the course of sarcoidosis appears to be similar in Philadelphia and in northern Europe. Although the prognosis appears to be less favorable in Negro patients and in those patients with many systems involved, analysis indicates that cutaneous involvement is the feature most closely associated with progressive and fatal sarcoidosis (after authors' summary).—T. H. Noehren

The Role of Splenectomy in Sarcoidosis. J. Bertino and R. M. Myerson*. *Arch Intern Med*, August, 1960, 106: 213–217. (*V.A. Hosp., Philadelphia, Penn.)

Despite the frequent involvement of the spleen in sarcoidosis, signs and symptoms due to the involvement of this organ are relatively infrequent. Splenectomy for splenic sarcoidosis is indicated in case of rupture of the spleen, when there is abdominal discomfort due to splenomegaly, or in the presence of persistent thrombocytopenia or pancytopenia. Neutropenia without severe agranulocytosis is probably not an indication for splenectomy. Acquired hemolytic anemia, especially when unassociated with splenomegaly, may not improve after surgery. Two cases are presented illustrating the beneficial long-term results of splenectomy. The pathogenesis of the hematologic abnormalities associated with splenic sarcoidosis are unknown, and further study is indicated (Authors' summary).—W. J. Steininger

Five Cases of "Alveolar Cell" Tumor of the Lung. J. C. Roberts*, R. G. May, and E. Nassau. *Tubercle*, August, 1960, 41: 257–267. (*Harefield Hosp., Middlesex, England)

Five cases of "alveolar cell" tumor of the lung are presented. The disease may sometimes be diagnosed by combination of the clinical picture, roentgenographic appearances, sputum examination, and biopsy. A frequent misdiagnosis is pulmonary tuberculosis. One case was remarkable for the quantity and nature of the sputum. It is noteworthy that when malignant cells were found in the sputum all of the pathologists who saw them regarded them as distinctly unusual in appearance. The lipoid-laden cells in this case have been described as an occasional feature of this disease. The disease is fatal. The condition appears to be multicentric in origin and surgery has apparently only palliative value.—M. J. Small

TUBERCULOSIS, PULMONARY

Current Status of Pulmonary Silicosis with or without Tuberculosis (in Japanese). M. Kato*. *Jap J Chest Dis*, August, 1960, 19: 527–532. (*Ministry of Labor, Tokyo, Japan)

Roentgenographic examination of workers exposed to mineral dust has been required by law since 1955, and 342,338 workers were examined voluntarily or in groups up to the end of March, 1958. The number of patients falling into the first, second, third, and fourth degrees of pulmonary silicosis were 32,208, 3,838, 2,150, and 3,237, respectively, and the total number of patients was 41,433. A new pneumoconiosis law passed the Diet and has been in effect since April 1, 1960. By this law, workers exposed to any kind of mineral dust are required to have their lungs examined roentgenographically, and they are protected medically and economically in case pulmonary lesions are found. Changes in diagnostic criteria and roentgenographic gradings may ensue in the near future. Most important of all of the factors brought out by this law is the knowledge that 98 per cent of the silicosis patients who require active treatment have the complication of pulmonary tuberculosis. —I. Tateno

Skin and Buccal Pigmentation, Pulmonary Tuberculosis and Steatorrhea. A. W. Lees*. *Brit J Dis Chest*, July, 1960, 54: 272–276. (*Ruchill Hosp., Glasgow, Scotland)

A case is reported in which steatorrhea was associated with skin and buccal pigmentation, asthenia, low blood pressure, a positive Kepler test, and pulmonary tuberculosis. Investigations showed that the adrenal cortex could nevertheless respond adequately to corticotropin stimulation. Attention is drawn to the fact that on occasion the presenting features of idiopathic steatorrhea may easily be mistaken for those of Addison's disease (after author's summary)—M. J. Small

The Risk of Tuberculosis in Adolescents and Young Adults. A Critical Study of the Protective Value of Spontaneous Allergy (in French). P. Fredour, J. Duhamel, M. Serrise, P. Courday, and P. Cassaigne. *Rev Tuberc (Par)*, May–June, 1960, 24: 678–693.

A study was made of the risk of tuberculosis in relation to allergy or anergy to tuberculin in a group of high school and college students between the ages of 16 and 18 in Bordeaux. After an observation period of 8 years (1945–1953), it was found that cases of tuberculosis of all forms were not more numerous among the nonreactors. However, considering only so-called "tertiary tuberculosis" of the phthisic type, there were larger

numbers in the allergic group; the difference was statistically significant. The risk of developing tuberculosis is higher in nonreactors if the general prevalence of tuberculosis is high; it is higher in tuberculin-positive individuals in a population with low incidence of tuberculosis (allergy due to BCG is not considered in this context).—V. LEITES

Spontaneous Paralysis of the Phrenic Nerve: With a Special Reference to Chronic Pulmonary Tuberculosis. S. K. GUPTA*. *Brit J Dis Chest*, July, 1960, 54: 283–289. (*Whittington Hosp., London, England)

Eight cases of paralysis of the phrenic nerves are described. All of these patients had been suffering from chronic pulmonary tuberculosis. The possible mechanism of this association is discussed and special stress is laid on postinflammatory fibrosis and chronic mediastinitis. One of the patients described had recurrent laryngeal nerve palsy in addition to ipsilateral hemidiaphragmatic paralysis. The literature is reviewed (after author's summary).—M. J. SMALL

TUBERCULOSIS, NONPULMONARY

Treatment of Nonhealing Tuberculous Cervical Fistulas (in German). H. MIEGL*. *Wein Med Wschr*, March, 1960, 110: 224–226. (*Grieskirchen, Austria)

The case history is presented of a 76-year-old woman who was treated for fistulous cervical lymphadenitis with streptomycin, isoniazid, and locally administered PAS. Secretions obtained from the fistulas were positive for acid-fast bacilli by smear, culture, and animal inoculation; there was no specific pulmonary process present. In 3 months of treatment, no significant improvement was observed. A suppository charged with 250 electrostatic units of thorium X was then inserted in each of the 3 fistulas, sealed hermetically, and timed separately. In 3 weeks, the patient was discharged as cured, and she has been asymptomatic for 3 years. Four other patients with the same diagnosis who did not improve on conventional antimicrobials were treated with complete success with this method modified by employing only 125 units in 1- to 2-week intervals. The thorium X employed is a radioisotope with a half-life of 78 hours. —Z. VIRÁGH

Histocytologic Examination and Biopsy of Lymph Nodes. Their Value in the Diagnosis of Lymphadenopathies, Particularly in Hodgkin's Disease (in French). R. PICARD*, J. KERNEIS, Y. BRUNEAU, and A. GORDEEFF. *Presse Med*, June 22, 1960, 31: 1203–1206. (*Nantes, France)

Used separately, biopsy and puncture of lymph nodes may often lead to the diagnosis of lymphadenopathies, although both procedures may fail to reveal the diagnosis. However, the combined evaluation of the results obtained by both methods has increased the percentage of positive results. The authors have used this combined method especially in Hodgkin's disease. It has also proved useful in the diagnosis of lymph node sarcoma.—E. LYON

Congenital Tuberculosis: Report of a Case. S. F. DAVIS*, S. C. FINLEY, and W. K. HARE. *J Pediat*, August, 1960, 57: 221–224. (*U. of Alabama Med. Center, Birmingham)

One hundred and fifty-seven cases of congenital tuberculosis have been reported in the American and foreign literature. Another patient who has thus far survived is here reported. This patient fulfills the majority of the criteria for a diagnosis of congenital tuberculosis: (*1*) the tuberculous nature of the lesion was proved by demonstration of acid-fast bacilli on microscopy of lymph nodes and by cultural growth of tubercle bacilli from the lymph node and bone marrow, (*2*) the child developed signs of illness on the 8th day of life, and abnormal physical findings appeared on the 22nd day, and (*3*) the child was immediately separated from the mother after delivery and has had no further contact with her. The infant has been treated with isoniazid and streptomycin. Calcifications in the spleen and liver were first apparent roentgenographically at the age of 4 months. The tuberculin skin test has remained negative, which is in keeping with other reported cases. At the present time, the relationship between cutaneous allergy and tuberculous disease is not clear. —M. J. SMALL

THORACIC SURGERY

Recurrent Bronchial Stricture: Review of Literature and Report of a Case Successfully Repaired. K. C. SZE*. *Ann Surg*, July, 1960, 152: 160–166. (*V. A. Hosp., Bronx, N. Y.)

A case is reported of recurrent bronchial stricture successfully treated by resection of the stenotic area and bronchotracheal anastomosis. Nine other cases of recurrent bronchial stricture have been collected, making a total of 10 cases. Tuberculous tracheobronchitis was the etiology in 6 cases, and automobile accidents in the remaining 4. The suture materials, the significance of tension at the suture line, the importance of postoperative bronchoscopy, and the effectiveness of bronchoscopic dilatations are considered.—M. J. SMALL

Pulmonary Alveolar Cell Carcinoma (in Roumanian). A. Eskenasy* and P. Nicolescu. *Ftiziologia*, January–February, 1960, 1: 67–74. (*Institute de Ftiziologie, Bucureşti, Roumania)

Four cases are reported with the known features of the disease. The diagnosis was established by histopathologic examination. One patient had surgery for hydatid cyst; the resected specimen revealed alveolar cell carcinoma. The postoperative course of this patient was good. The other 3 patients died, 1 of them 1 year after lobectomy for a neoplastic process.—Z. Virágh

Surgical Treatment of Bullous Emphysema. J. L. Kee, Jr.*, R. R. Shaw, and D. L. Paulson. *JAMA*, July 9, 1960, 173: 1093–1097. (*Baylor U., Dallas, Texas)

Operations were performed on 37 patients with bullous emphysema. The results were excellent in 8 and improvement was achieved in 18, while 3 patients showed only temporary relief. Five patients died of progressive disease and 3 were lost to follow-up observation. Good results from surgical therapy can be expected from conservative operations performed on symptomatic patients who have localized disease occupying at least the area of one lobe.—H. Abeles

Gastro-Pleural Fistula as a Complication of Esophageal Hiatal Hernia. A. M. Markowitz* and F. P. Herter. *Ann Surg*, July, 1960, 152: 129–134. (*College of Physicians and Surgeons, Columbia U., N. Y.)

Two cases are presented of gastropleural fistulas secondary to perforation of the stomach in esophageal hiatal hernia. In both cases the hiatal hernia was essentially asymptomatic prior to the perforation, and in both this complication proved to be fatal. While rare, perforation of the herniated stomach into the pleural cavity should be added to the growing list of complications of the untreated hiatal hernia. Possible etiologic factors leading to the formation of a gastropleural fistula are reviewed.—M. J. Small

Surgical Management of Focalized Pulmonary Histoplasmosis. J. H. Forsee* and M. Pfotenhauer. *JAMA*, June 25, 1960, 173: 878–883. (*Walter Reed General Hosp., Washington, D. C.)

In 57 patients who underwent thoracotomy for removal of a coin lesion, a diagnosis of pulmonary histoplasmoma was established. Postoperative complications required thoracoplasty in 1 case, segmental resection for pulmonary hematoma in a second, and drainage of a chest wall abscess which contained *H. capsulatum* in a third case. Two patients had late reactivation of their disease.
—H. Abeles

Pneumomediastinum and Subcutaneous Emphysema in Status Asthmaticus, Requiring Surgical Decompression. B. McNicholl*. *Arch Dis Child*, August, 1960, 35: 389–392. (*Regional Hosp., Galway, Scotland)

An 8-year-old boy who had been subject to chronic asthma developed subcutaneous emphysema and pneumomediastinum during an exceptionally severe attack of status asthmaticus. His critical condition was not alleviated by epinephrine (Adrenalin®) or by intramuscular cortisone, and he developed pulsus paradoxicus. Incisions above the clavicle and exploration with a sinus forceps released air which was under pressure. This led to marked relief of the symptoms. Two further cases of pneumomediastinum and subcutaneous emphysema are briefly mentioned. These occurred in 2 siblings, aged 4 and 5 years, in the course of bronchopneumonia; no surgical measures were necessary.—J. Lorber

Experimental Repair of Tracheal Defects with Gall Bladder Mucosa. J. Bell*. *Dis Chest*, August, 1960, 38: 140–147. (*U. of Washington School of Med., Seattle)

There has been no really satisfactory solution to tracheal reconstruction with either prosthetics or host tissues. The most promising results have utilized inert materials for support of the lumen, with or without the addition of covering tissues. Tissue autografts with osteo- or chondrogenic induction capacity offer the possibility of permanent rigid-tube replacement of cylindrical tracheal defects. New cartilage and bone formation may be consistently induced in the paratracheal area by implantation of gall bladder mucosa.—E. A. Rouff

Pulmonary Resection in So-called Poor Risk Tuberculous Patients with Expected Vital Capacity Values under 50 Per Cent of Normal (in Japanese). H. Hasegawa*, A. Asaumi, T. Hosoda, A. Katsura, S. Matsui, T. Tezuka, B. Mimura, M. Uragami, A. Nakano, and J. Watanabe. *Jap J Thor Surg*, August, 1960, 13: 644–649. (*National Nakano Sanatorium, Tokyo, Japan)

The writers report the results of pulmonary resection in 32 "poor risk" cases of pulmonary tuberculosis with expected vital capacity values of less than 50 per cent. Although no patients died immediately following the operation, 2 died within 3 months after, and 3 died more than 3 months after operation. Death in each case was attributed to right heart failure, as judged from clinical and autopsy findings. The right heart failure was apparently induced by poor pulmonary vascular beds, and was accelerated by anoxia due to postoperative ventilatory insufficiency. It was considered neces-

sary to maintain the patient's expected vital capacity above 35 per cent of normal. Patients with an air velocity index higher than 1 had better prognoses than those with indices lower than 1. Tracheotomy with cuffed cannula was very useful for prevention of anoxia due to hypoventilation, especially in poor risk patients. Methods for prevention of right heart failure due to pulmonary hypertension should be established in the future. —I. TATENO

Present Status of Indications for Surgery in Pulmonary Tuberculosis (in German). G. SALZER*. *Wien Med Wschr*, February, 1960, 110: 187–188. (*Wolkersbergenstrasse 1, Vienna, Austria)

In Austria, indication for lung surgery is decided by the chest physician, but the type of operation is the responsibility of the surgeon. Pneumolysis might be performed in cases of relatively fresh, not-too-large cavities in the apical area of 1 lung. Thoracoplasty is indicated in older persons with a shrinking, small cavitary process in the upper lobe, which can be brought under control by removing not more than 5 or 6 ribs. In the majority of cases, pulmonary resection is the operation of choice, with due consideration of these factors: what remains that is still operable, and which section has to be removed. For the first factor, the following may influence the decision for surgery: bilateral processes; unfavorable pulmonary function; coexisting ulcerative bronchial tuberculosis resistant to treatment; uncontrollable hemoptysis; a serious condition caused by tuberculosis toxicity; decreased susceptibility to antituberculous drugs; diabetes; peptic ulcer; heart failure; chronic nephritis; lues; unfavorable hematologic condition. In general, the indications are similar to those followed in the U. S. and the Netherlands. The second factor—what sections have to be removed—refers to coin lesions, including tuberculomas, which should be removed only if there is sign of activity, such as change in size or development of cavity. —Z. VIRÁGH

Recidivation after Pulmonary Resection for Tuberculosis (in German). J. KRAAN*. *Beitr Klin Tuberk*, March, 1960, 122: 18–25. (*Sanatorium "Beatrixoord," Appelscha, Netherlands)

In the period from 1948–1957, there were 121 pneumonectomies, 259 lobectomies, and 697 segmentectomies performed in the Beatrixoord Sanatorium. Recidivation occurred in 5 patients (4.1 per cent) with pneumonectomies, in 29 (10.2 per cent) with lobectomy, and in 40 (5.7 per cent) with segmental resection. The possibility of recidivation is very slight if the patient is treated for a sufficient length of time before and after the operation.—Z. VIRÁGH

Percutaneous Needle Biopsy in Clinically Inoperable Pulmonary Tumors. F. I. MILLER*. *US Armed Forces Med J*, August, 1960, 11: 858–871. (*Walter Reed Gen. Hosp., Washington, D. C.)

Percutaneous biopsy of the lung was performed with a Vim-Silverman needle in 10 consecutive patients who had clinical diagnoses of pulmonary tumor deemed inoperable for cure or palliation. The needle biopsy gave tissue confirmation of pulmonary malignancy in all 10 patients. This, in effect, exempted each from a diagnostic thoracotomy. There were 3 complications: in 2 patients a small, quickly resolving pneumothorax, and in 1 patient a small, transient hemoptysis. With careful preparation, needle biopsy of lung tumors is relatively simple and safe. The procedure is of value in confirming the diagnosis of malignant pulmonary lesions clinically considered inoperable (after author's summary).—C. H. PIERCE

ROENTGENOGRAPHY

Disturbance of Cerebral Function Following Angiography of the Pulmonary Vessels (in German). H. SCHOLTZE*, H. EHRHARDT, and E. W. FÜNFGELD. *Tuberkulosearzt*, July, 1960, 14: 436–450. (*Sanatorium Sonnenblick, Marburg/Lahn, Germany)

Neurologic and electro-encephalographic examinations were made before and after angiography of the pulmonary vessels in 46 patients with severe pulmonary tuberculosis. An average of 170 ml. of Urografin® (76 per cent solution of a triiodized compound) was used. Within 2 to 7 days after the angiography, 8 patients developed changes in the the electro-encephalogram (EEG) (slowing of rhythm), while in 2 patients the pre-existing alterations in EEG became more marked. No correlation could be established between the development of these disturbances and the age of the patient, the duration and extent of disease, or the volume of contrast medium used. Follow-up tracings taken in the course of several months showed a gradual return to a normal, or nearly normal, rhythm. Neurologic examination did not reveal any evidence of cerebral lesions.—J. HAAPANEN

Coarctation of the Aorta. Relation between Roentgenologic and Hemodynamic Findings. C. E. GUDBJERG* and O. PETERSEN. *Radiology*, September, 1960, 75: 399–405. (*Rigshospitalet, Copenhagen, Denmark)

In a series of 56 cases of coarctation of the aorta, direct measurements of the intra-arterial pressure proximal and distal to the stenosis were obtained

in 35 patients. These measurements were compared with the width of the ascending and descending aorta. No correlation between a high pressure above the stenosis and dilatation of the ascending aorta could be demonstrated. In 4 of 20 patients with pronounced dilatation of the ascending aorta the intra-arterial pressure proximal to the site of the coarctation was normal, and no collaterals or notching of the ribs could be demonstrated. In these 4 cases the coarctation had no hemodynamic consequences and they are believed to represent "pseudocoarctation," the pathogenesis of which is obscure. Presumably, it is a systemic disease of the vascular wall.—W. J. Steininger

Bronchography in Pulmonary Tuberculosis. N. C. Elphinstone* and S. Z. Kalinowski. *Brit J Dis Chest*, July, 1960, 54: 277–282. (*St. Wulstan's Hosp., Malvern, Worcestershire, England)

Seven hundred bronchograms done on patients with pulmonary tuberculosis are reviewed. Although the percentage of abnormal bronchograms is high (90 per cent), the value of bronchography in pulmonary tuberculosis is very limited. Its main use is in localization of disease before operation, although it does not seem to be superior to lateral tomography for this. The changes were not specific for tuberculosis. Bronchitic changes were noted in a high proportion of cases. It is suggested that a follow-up study of these patients would be interesting to see whether bronchograms will revert to normal after arrest or cure of tuberculosis.
—M. J. Small

New Dimensions in Chest Radiography. L. Etter* and L. Cross. *Dis Chest*, August, 1960, 38: 124–130. (*School of Med., U. of Pittsburgh, Penn.)

Consideration of the replacement of 14- × 17-inch roentgenographic film by the 14- × 14-inch size for chest films is presented: the financial saving on film cost alone is considerable (almost 15 cents per film). The reduction in radiation dosage is of more importance. The minimal amount of "retooling" for utilizing the smaller film is detailed, and is actually not too considerable.—E. A. Rouff

Solitary Coin Lesions of the Lung. W. O. Pischnotte* and B. P. Sammons. *JAMA*, August 6, 1960, 173: 1532–1535. (*U.S. Naval Hosp., St. Albans, N. Y.)

Six malignancies were found in a series of 95 patients with coin lesions. The need for thoracotomy in almost all cases of coin lesions is stressed. The presence of calcium is regarded as a good sign of a benign condition, but is not proof of the innocuous nature of the lesion.—H. Abeles

Respiratory Kymography in Acute Abdominal Conditions. S. Schmidt*. *Acta Radiol (Stockh)*, July, 1960, 54: 49–68. (*Karolinska Sjukhuset, Stockholm, Sweden)

Roentgen kymographic investigation of diaphragmatic and costal movements in various acute abdominal conditions is reported. The technique, using 30- × 40-cm. films, is described in detail. Eighty-seven acute abdominal cases as well as 30 normal cases were studied. Considerable restricted diaphragmatic movement was found on the affected side. Slight or moderate impairment of costal respiration was reported in practically all severe abdominal conditions. The diaphragmatic waves in severe cases were plateau shaped. Such waves indicate peritonitis or peritoneal irritation of a local nature. Kymographic appearances often help differentiate abdominal from thoracic conditions. Right or left side abdominal involvement is predictable. The procedure is reported to be a valuable single and rapid method of examination with little or no discomfort to even the most gravely ill patient.—E. Shabart

The Sickle-Shaped, Right-Sided, Paramediastinal Air Space in Cases of Shrunken Right Upper Lobe (in German). E. Bürgel* and H. G. Oleck. *Fortschr Roentgenstr Nuklearmed*, August, 1960, 93: 160–163. (*Wenckebach Hosp., Berlin, Germany)

The chest roentgenogram of a patient with carcinoma of the right upper lobe bronchus showed a sickle-shaped, laterally convex air space between the mediastinum and the shrunken right upper lobe. At autopsy, a special technique was used to study the topographic relationship of the intrathoracic organs. The unopened thorax was frozen *in toto*, and horizontal, frozen cross-sections were examined. At the level of the fourth dorsal vertebra, the cross-sections demonstrated an interposition of the lower lobe between the mediastinum and the shrunken upper lobe corresponding to the above described, roentgenographically observed air space.—H. Abeles

***Pneumocystis Carinii* Pneumonia.** C. W. Rowe*. *Radiology*, August, 1960, 75: 257–261. (*U. of Texas Medical Branch, Galveston)

Pneumocystis carinii pneumonia is apparently still rare in the United States, but it has reached epidemic proportions in Europe in the last decade. The seventh unequivocally proved death from this cause in the United States, occurring in an infant of 6 months, is presented. The disease occurs endemically or epidemically within hospital nurseries and seems to have a predilection for premature or debilitated infants, although it has

been recorded several times in adult subjects. The discrepancy between the severity of dyspnea and the paucity of physical findings is typical. Antemortem diagnosis of *Pneumocystis carinii* pneumonia is difficult, but roentgenographic findings are consistent and helpful in making a presumptive diagnosis. They may precede clinical signs and symptoms. Pulmonary infiltration apears hazy-to-mottled, extending out from the hilar region in a fanlike distribution which may lead to confusion with pulmonary edema. The process may spread eventually to the periphery so that no air space can be seen on the films. Interstitial emphysema is frequently diffuse, markedly developed, and varied in form.—W. J. Steininger

CHEMOTHERAPY

Severe Carbon Dioxide Intoxication—Treatment with Organic Buffer (Trihydroxymethylaminomethane). F. Manfredi*, H. O. Sieker, A. P. Spoto, and H. A. Saltzman. *JAMA*, July 2, 1960, 173: 999–1001. (*Duke U., Durham, N. C.)

Infusions of an organic carbon dioxide buffer, trihydroxymethylaminomethane (THAM), were given to 3 patients with chronic lung disease, superimposed infection, acute respiratory insufficiency, and carbon dioxide narcosis. In 2 cases there was marked improvement in the respiratory acidosis and hypercapnia. The third patient died. No side effects were noted.—H. Abeles

Corticosteroids in the Treatment of Hemoptysis (in Roumanian). N. Constantinescu*, S. Krauss, M. Constantinescu, and C. Ciulavu. *Ftiziologia*, January–February, 1960, 1: 41–45. (*Sanatoriu TBC Solca, Roumania)

Of 50 patients treated with customary doses of various corticosteroids, moderate or severe hemoptysis was stopped in 36 (72 per cent) within 24 to 36 hours; in 10 patients (20 per cent) the result was not convincing, and 4 patients (8 per cent) failed to respond. No untoward effect was observed.—Z. Virágh

Amphotericin B in Bilateral Cavitary Histoplasmosis—Report of a Case with Two-Year Follow-Up. M. Sinn*. *JAMA*, July 16, 1960, 173: 1220–1222. (*Evansville, Ind.)

In a case of bilateral cavitary pulmonary histoplasmosis, intravenous therapy with 50 mg. of amphotericin B daily for about 90 days resulted in dramatic improvement. The patient has remained relatively well during the 2-year follow-up period, although she has residual disease with emphysema and fibrosis.—H. Abeles

A Method of Titration of the Concentration of Isoniazid in Human Serum (in Japanese). R. Okamoto*, R. Utsunomiya, and K. Hiruizumi. *Kekkaku*, August, 1960, 35: 543–546. (*Muramatsu Seriranso Sanatorium, Ibaraki Prefecture, Japan)

The authors cultured tubercle bacilli in Kirchner's agar medium containing 0.25 per cent agar in test tubes in the upright position. The tubercle bacilli usually grew on the surface of the medium, forming a band of colonies. When solutions containing isoniazid were overlayered on top of the medium, the zone of the growth of tubercle bacilli dropped below the surface of the medium. The distance of submersion of the growth zone was 20 mm. for 1γ per ml., 10 mm. for 0.5γ per ml., 5 mm. for 0.25γ per ml., and 2 mm. for 0.125γ per ml. of isoniazid concentration, respectively. The distance varied as the concentration of the inoculum varied. Any of the isoniazid-susceptible tubercle bacilli isolated from untreated patients could be used as a test strain. The tubercle bacilli which were cultivated in Dubos liquid medium and used as inoculum to Kirchner medium grew into a visible band of colonies in 5 to 6 days. The determination of the concentration of isoniazid in the serum was possible within this period, which was much shorter than the other method which required 2 weeks of incubation.—I. Tateno

Genetic Control of Isoniazid Metabolism in Man. D. A. Price Evans*, K. A. Manley, and V. A. McKusick. *Brit Med J*, August 13, 1960, No. 5197: 485–492. (*Johns Hopkins U. School of Med., Baltimore, Md.)

Plasma isoniazid concentrations have been determined chemically 6 hours after drug ingestion in 484 subjects. The frequency distribution curve of these concentrations is bimodal. Individuals are divided by the antimode into rapid and slow inactivators of isoniazid. There is no heterogeneity for these characters due to sex or age and, on the numbers studied, the distribution of phenotypes appears to be the same in American Negroes as in whites. The number of subjects studied is too small to determine whether tuberculosis affects one phenotype more than another; 267 of the subjects studied constituted 53 white families. The family data support the hypothesis that the slow-inactivator character is recessive. A "dosage" effect of the allele controlling the dominant character is demonstrable in that there is no significant difference between the mean plasma isoniazid concentration of recognizable heterozygotes and the mean value of all other rapid inactivators. Possible reasons for the maintenance of the polymorphism are discussed, as are the therapeutic implications of this genetically-determined isoniazid inactivator character (Authors' summary).—E. A. Riley

The Management of Cryptococcal Meningitis. M. J. Fitzpatrick* and C. M. Poser. *Arch Intern Med*, August, 1960, 106: 261–270. (*U. of Kansas Med. Center, Kansas City)

Experience in the management of 6 patients with cryptococcal meningitis in the past 4 years is presented. In 4 patients amphotericin B therapy was used; in 3 the drug was administered intravenously, and all survived. Administration of intravenous amphotericin B on alternate days in doses of 1 mg. per kg. of body weight is the treatment of choice. At least 4 weeks of treatment are necessary. Frequently, intravenous treatment spaced over 2 to 3 months will prove beneficial. The need for intrathecal augmentation of amphotericin B treatment in cryptococcal meningitis is still undecided. The role of excisional surgery and chemotherapy in the management of cryptococcosis is discussed. —W. J. Steininger

Amphotericin B Therapy in Torula Meningitis. M. J. Barrash* and M. Fort. *Arch Intern Med*, August, 1960, 106: 271–274. (*Michael Reese Hosp., Chicago, Ill.)

Amphotericin B is the drug of choice in torula meningitis, and may prove to be curative in some cases. A case of torula meningitis responding rapidly to amphotericin B is described. Some observations concerning details of treatment with this agent are presented, and an alert is sounded as to a possible toxic effect on the blood-forming organs. —W. J. Steininger

The Relationship of Food Intake to the Absorption of Potassium Alpha-Phenoxyethyl Penicillin and Potassium Phenoxymethyl Penicillin from the Gastrointestinal Tract. G. A. Cronk*, W. B. Wheatley, G. F. Fellers, and H. Albright. *Amer J Med Sci*, August, 1960, 240: 219–225. (*Syracuse U., New York)

Oral doses of potassium alpha-phenoxyethyl penicillin (syncillin) and potassium phenoxymethyl penicillin (penicillin V) were administered to groups of human volunteers at varying intervals before, during, or after meals. It appears that orally administered syncillin is absorbed better from the gastrointestinal tract than is penicillin V whether the subject is fasting or has ingested food before, with, or after the antimicrobial. The experiments suggest that to obtain maximal penicillin serum concentrations, both preparations should be administered before meals. —W. J. Steininger

Treatment of Pneumonia and Other Serious Infections. M. Finland*. *New Engl J Med*, August 4, 1960, 263: 207–221. (*Boston City Hosp., Mass.)

Blood-stream invasion by the 4 common coliform organisms, *Esch. coli*, Proteus, *Ps. aeruginosa*, and *A. aerogenes*, as well as invasion due to enterococci, has increased markedly as a cause of morbidity and mortality since the introduction of modern antimicrobial drugs. The figures for these 5 infections indicate about a sixfold increase in the number of cases and a seven-fold increase in the number of deaths. Collectively, they accounted for only about 12 per cent of all bacteremic infections and 9 per cent of all deaths from such infections in 1935, but they have constituted more than ⅓ of such cases and about ½ of the deaths among bacteremic patients since antimicrobials came into wide use. Whereas pneumococci and hemolytic streptococci were formerly the most common causes of bacteremic infections and accounted for more than ½ the cases and nearly ¾ of all deaths from such infections, they now account for less than ⅓ of the cases and less than ⅛ of the deaths. In sharp contrast are the changes in *Staph. aureus* and the organisms that have generally been considered normal constituents of the bowel flora—namely, *Esch. coli*, *A. aerogenes*, Proteus, Pseudomonas, and Enterococcus—which for brevity and convenience may be lumped together under the term enterobacteria, although it may not be taxonomically precise. In 1935, *Staph. aureus* accounted for slightly more than 1 of every 5 cases and less than 1 of every 5 deaths from bacteremic infections, whereas in 1957 it accounted for about ⅔ of both the cases and the deaths. What are here called normal enterobacteria accounted for less than 1 in 8 cases and 1 in 11 deaths before the chemotherapeutic era; now they account for more than ⅓ of all the cases and ⅔ of the deaths from bacteremic infections.

The picture of the serious bacterial infections, as reflected in blood-stream invasion, is not a pleasant one to contemplate. It may be an expression of 2 underlying biologic principles. One of these has to do either with adaptation—that is, the acquisition of resistance to the antibacterial agents—or with the selection of resistant variants by exposure to them. The other is the result of changes in ecology of the various bacterial species that constitute the "normal bacterial flora" and the interrelations of these bacteria. The excessive and almost universal use of antimicrobial agents, particularly in the hope of preventing unknown or ill-defined infections, may be largely responsible for the situation in hospitals. Increasing laxity in the application of strict aseptic techniques and principles for avoiding infection and cross-infection in surgery, in nurseries, and in general wards in which infections are being treated, is undoubtedly a major contributing factor. Perhaps changes in the types of patients being encountered—the increasing

proportions of the aged, the debilitated, and those with chronic bronchopulmonary or urinary-tract diseases, who previously died earlier in the course of their illnesses—are equally important.
—M. J. Small

Treatment of Pneumococcal Pneumonia with a New Penicillin: Clinical Observations in 25 Patients. P. C. Bunn* and R. Knight. *Amer J Med Sci*, August, 1960, 240: 192–196. (*State U. of N. Y. Upstate Med. School, Syracuse)

Twenty-five patients with pneumococcal pneumonia were treated with the new partially synthesized potassium alpha phenoxyethyl penicillin (syncillin). Results were satisfactory in all: there were no deaths, no purulent complications, and no untoward reactions from the drug itself. Dosage schedule in the last 11 of the group was 250 mg. orally, 3 times daily, each dose given between meals. Results with these dosages were as good as in the first 14 who were treated with larger amounts. If prescribed properly, syncillin is a fine preparation, as effective as any product for the treatment of pneumococcal pneumonia.
—W. J. Steininger

Effect of Pyrazinamide on Antimicrobially Active Serum Isoniazid. D. Levy*, S. Duysak, B. Zylberberg, J. Haapanen, W. Russell, Jr., and G. Middlebrook. *Dis Chest*, August, 1960, 38: 148–151. (*National Jewish Hosp., Denver, Colo.)

Previous studies have shown that concurrent administration of PAS with isoniazid raised the serum concentration of active isoniazid when measured 6 hours after the ingestion of the loading dose. It is believed that this may account for the greater efficacy of two drugs over isoniazid alone. Similar studies using pyrazinamide with isoniazid showed no effect on the serum concentrations of isoniazid.
—E. A. Rouff

Studies on the Effect of Alpha-Ethyl-Thioisonicotinamide (1314 Th) on Kanamycin-resistant Tubercle Bacilli (in Japanese). T. Toda*, K. Kutune, Y. Hagiwara, and K. Watanabe. *Jap J Chest Dis*, August, 1960, 19: 590–593. (*Kyushu U., Hukuoka, Japan)

Alpha-ethyl-thioisonicotinamide (1314 Th) inhibited the growth of a kanamycin-resistant H37Rv strain of tubercle bacilli at a concentration of 0.8γ per ml. *in vitro*, and completely inhibited the growth at 1.0γ per ml. Its inhibitory action was superior to that of streptomycin. Mice infected with kanamycin-resistant tubercle bacilli were treated daily with 1 mg. of 1314 Th. The result was superior to the result of treatment with the same dose of streptomycin and was equal to that obtained with 0.1 mg. of isoniazid daily. 1314 Th is expected to be useful in the treatment of pulmonary tuberculosis with kanamycin-resistant tubercle bacilli or other forms of intractable tuberculosis.—I. Tateno

Treatment of Tuberculosis in Children. A Statement by the Committee on Tuberculosis and Respiratory Diseases in Children*. *J. Pediat*, August, 1960, 57: 290–293. (*American Thoracic Society, Nat. Tuberc. Ass'n., N. Y.)

Isoniazid should be a part of every regimen for treatment of active tuberculosis; the usual dosage level is about 15 mg. per kg. daily, taken in 2 or 3 doses. Pyridoxine has not seemed to be a necessary additional drug for the prevention of neuritis in children, although it may be necessary in adolescents. Para-aminosalicylic acid is the most commonly used second drug when a combination regimen is employed. Streptomycin is not used except under special conditions. Dihydrostreptomycin should not be used in pediatric practice. "Triple-drug therapy" should be reserved for the more serious forms of disease. Corticosteroids should be used for the infant or young child who is dangerously ill in order to support him until antimicrobial drugs have an opportunity to produce some effect. The exact optimal duration of therapy is not known, but the usual duration of drug therapy is at least 1 year and, preferably, 18 or even 24 months. All patients with tuberculous meningitis and miliary tuberculosis should receive triple-drug therapy, with streptomycin given daily (at least for a few weeks), and isoniazid in comparatively high dosage. If tuberculin conversion is known to have occurred within 1 year prior to the time of examination, all children up to 14 years should be placed on treatment. The treatment should be continued for at least 1 year and possibly longer. Isolation and bed rest are not necessary. When the date of conversion of the tuberculin reaction is unknown and the patient has no signs or symptoms, it is thought that for maximal safety all patients less than 36 months old should be treated for at least 1 year. The larger the initial tuberculin reaction the greater the risk of that child's developing active tuberculous disease. Any tuberculin-positive child who is to receive steroid treatment for another disease should receive concurrent antituberculosis treatment. A child with a positive tuberculin reaction should receive antituberculosis therapy for at least 4 weeks in the event of an attack of measles.—M. J. Small

The Initiation of Chemotherapy in Pulmonary Tuberculosis in West Africans: Hospital and Ambulant Treatment Compared Using Streptomycyclidine–Isonicotinic Acid Hydrazide. W. J. Bell*. *Brit J Dis Chest*, July, 1960, 54:

247–254. (*TB Research Unit, West African Council for Med. Research, Accra, Ghana)

A controlled study has been undertaken to determine the usefulness of initial short-term hospitalization in the chemotherapy of pulmonary tuberculosis in West African males. Thirty-three patients were treated in hospital for a period of 12 weeks, and 56 were treated entirely on an outpatient basis for the same period. Patients were allocated to inpatient and outpatient treatment groups irrespective of clinical condition. The inpatients were given daily injections of streptomycyclidineisonicotinylhydrazine (Streptohydrazid®). Patients were allow up for toilet purposes and also to sit up for 1 hour daily. Otherwise, strict bed rest was enforced. A standard African hospital dietary regimen was supplied. The outpatients were required to report daily, for a period of 12 weeks, for injections of Streptohydrazid. No restriction was placed on their activity, and if a patient felt well enough to continue work, he was free to do so. Each was encouraged to take his normal diet, and no supplementary feeding was given. No significant differences in the rates of clinical improvement, weight gain, roentgenographic change, or sputum conversion were demonstrated at the end of 3 months' treatment.
—M. J. Small

Results of the Treatment of Tuberculosis Before and Since the Introduction of Chemotherapy. H. E. Pugsley*, E. A. Allen, O. T. Cheung, H. S. Coulthard, and G. L. Gale. *Canad Med Ass J*, August 27, 1960, 83: 424–429. (*Toronto Hosp. for Tuberc., Weston, Ontario, Canada)

Before the introduction of chemotherapy, the mortality rate for minimal active pulmonary tuberculosis was 5 per cent, for moderately advanced disease it was 20 per cent, and for far advanced disease, 70 per cent. Following the use of chemotherapy, the 5-year mortality rate at the Toronto Hospital for Tuberculosis was zero for minimal and moderately advanced disease, and 11 per cent for far advanced disease. The 140 patients in this series received triple-drug therapy for an average of 17 months. The sputum of all patients had converted after 18 months of chemotherapy, except in 2 patients who died within 6 weeks after starting chemotherapy. Some form of resectional therapy was necessary in 22 per cent of the group. Three years after the beginning of chemotherapy, 100 per cent of the minimal group, 97 per cent of the moderately advanced group, and 81 per cent of the far advanced group were inactive. The over-all relapse rate was 4 per cent. A comparison of patients with bone and joint tuberculosis treated before and after the introduction of chemotherapy indicates a 27 per cent death rate before the advent of chemotherapy and a zero death rate after introduction of chemotherapy. The average duration of immobilization for spinal tuberculosis was reduced from 20 months to 14 months, and for tuberculosis of the hip from 27 months to 15 months. The incidence of arthrodesis or grafting operations was reduced from 80 per cent to 33 per cent, and the incidence of relapse from 34 per cent to 6 per cent. Among 82 patients with renal tuberculosis who had neither chemotherapy nor nephrectomy, 58 per cent died of tuberculosis. Among 347 cases treated by nephrectomy without chemotherapy, 46 per cent died of tuberculosis. Adequate chemotherapy in a group of 163 cases resulted in a mortality of 1 per cent and a relapse rate of only 1 per cent.—E. A. Riley

Ethionamide ('1314') with Streptomycin in Acute Tuberculosis of Recent Origin in Uganda Africans: A Pilot Study. P. W. Hutton* and I. M. Tonkin. *Tubercle*, August, 1960, 41: 253–256. (*Mulago Hosp., Uganda, East Africa)

Ethionamide was given with streptomycin to 7 previously untreated Uganda Africans with acute rapidly progressive tuberculosis. Streptomycin was given in a daily dose of 1 gm. of streptomycin sulfate intramuscularly; 1 gm. of ethionamide was administered orally in 4 divided doses. Treatment was continued for 6 months. Clinical, bacteriologic, and roentgenographic improvement was observed in all cases. Three showed considerable improvement and 4 showed moderate improvement. Comparison of the roentgenograms at 6 months with those before treatment showed that the cavities had disappeared in 3 patients and were smaller in 3; in 1 patient the cavity was larger. One patient produced a single colony culture resistant to streptomycin, isoniazid, ethionamide, and PAS after 6 months. All previous cultures were susceptible. This was the only positive culture obtained in the 6 patients in whom cultures were obtained at 6 months. This pilot study suggests that further investigation of this drug combination is desirable. It suggests that streptomycin and ethionamide together give rise to less streptomycin resistance than would have been expected had streptomycin alone been used.
—M. J. Small

Pyrazinamide in the Treatment of Pulmonary Tuberculosis (in German). B. Van Dijk* and J. Kraan. *Beitr Klin Tuberk*, March, 1960, 122: 71–75. (*Sanatorium "Beatrixoord," Appelscha, Netherlands)

Of 125 patients treated with pyrazinamide in a daily dose of 2 gm. for at least 3 months, 78 showed improvement on roentgenogram; 46 of the 64 patients with positive acid-fast bacillary findings had reversal of infectiousness. The drug was administered together with 1 or more of the conventional antimicrobials. The best results were achieved in patients with the exudative form of tuberculosis; fibrous and old cavitary processes showed hardly any improvement. Resistance to the companion drugs did not influence the effectiveness of pyrazinamide. There were no serious symptoms referable to liver damage.—Z. VIRÁGH

The Results of Treatment with Streptomycin Plus Pyrazinamide in Patients with Active Pulmonary Tuberculosis Despite Prolonged Treatment with Isoniazid Plus PAS. S. VELU*, R. ANDREWS, S. DEVADATTA, W. FOX, C. RAMAKRISHNAN, and T. SUBBAIAH. *Indian J Tuberc*, June, 1960, 7: 84–94. (*Tuberculosis Chemotherapy Center, Madras, India)

A supervised drug-administration study in domiciliary patients in a community in which self-administration of drugs could not be depended upon has shown attainment of bacteriologically quiescent disease in 10 of 20 patients followed for more than 1 year. All had previously received adequate courses of isoniazid with PAS and were shown to have streptomycin-susceptible bacilli. Rapid conversion of the sputum within 3 months was demonstrated in all 10 successful cases. A high degree of streptomycin resistance was present in the patients whose response was unsatisfactory. Toxicity was not a problem with either drug.
—E. A. ROUFF

The Medical Letter*. (*136 East 57th St., N.Y.)

November 25, 1960, 2: 93–96: Antacids in ulcer therapy (sodium and calcium carbonate). Xylometrazoline hydrochloride (Otrivin®) in relief of nasal congestion.

December 9, 1960, 2: 97–100: Phenazocine (Prinadol®), a new analgesic-narcotic as substitute for morphine. Corticosteroid therapy in children; effects in viral infections. Toxicity of meprobamate (Miltown®, Equanil®).

PULMONARY PHYSIOLOGY

Measuring Bronchial Resistance (in German). M. JAEGER*. *Schweiz Med Wschr*, June 11, 1960, 90: 648–654. (*Medizinische Klinik, Universität Bern, Switzerland)

The resistance offered by the respiratory tract to air flow was determined in 25 normal and 48 asthmatic subjects using 2 methods simultaneously, namely, measuring the esophageal pressure and pressure at the mouth after interruption of air flow. The correlation between the resistance—calculated from the esophageal pressure—and the routine lung function tests reflecting bronchial obstruction proves that the esophageal method gives a real picture of the severity of bronchial obstruction. No good correlation was found by the interruption technique.—J. HAAPANEN

Effects of Breathing Carbon Dioxide upon the Pulmonary Circulation. A. P. FISHMAN*, H. W. FRITTS, JR., and A. COURNAND. *Circulation*, August, 1960, 22: 220–225. (*College of Physicians and Surgeons, Columbia U., N.Y.)

The effects on the pulmonary arterial blood pressure and flow of inhaling 5 per cent carbon dioxide in air were studied in normal man and in patients with chronic pulmonary emphysema. In the 5 control subjects, with an average increase in arterial P_{CO_2} of 6 mm. Hg (37 to 43) and a threefold increase in minute ventilation, both pulmonary arterial blood pressure and flow remained unchanged. In the 10 patients with chronic pulmonary emphysema with a similar increase in arterial P_{CO_2} (45 to 52) and a twofold increase in minute ventilation, there was a 14 per cent increase in cardiac output and a rise in pulmonary arterial mean pressure of 4 mm. Hg. In these patients an increment in pulmonary arterial pressure was invariably asociated with an appreciable increment in blood flow. The present study affords no support for the view that the breathing of air enriched with carbon dioxide elicits pulmonary vasoconstriction in either normal subjects or in patients with chronic pulmonary disease.—W. J. STEININGER

Effects of Acute Hypoxia on the Volume of Blood in the Thorax. H. W. FRITTS, JR.*, J. E. ODELL, P. HARRIS, E. W. BRAUNWALD, and A. P. FISHMAN. *Circulation*, August, 1960, 22: 216–219. (*College of Physicians and Surgeons, Columbia U., N.Y.)

The effect of acute hypoxia on the volume of blood in the thorax was studied by 2 different methods: the first entailed estimation of the central blood volume by the Stewart-Hamilton dye dilution technique; the second involved the use of a teeter-board. Both methods indicated that the volume of blood in the thorax is not altered appreciably by hypoxia.—W. J. STEININGER

Lung Compliance and Respiratory Resistance. L. EHRNER*. *Acta Med Scand*, July 15, 1960, 167: Supp. 353: 13–220. (*Karolinska Sjukhuset, Stockholm, Sweden)

Shunting Effect of Extreme Impairment of Pulmonary Diffusion. S. I. Said*, W. T. Thompson, Jr., J. L. Patterson, Jr., and D. L. Brummer. *Bull Johns Hopkins Hosp*, No. 5, 1960, 107: 255–261. (*Med. College of Virginia, Richmond)

The physiologic effects of extreme impairment of pulmonary diffusion have been analyzed in five cases of "alveolar-capillary block." The physiologic effects of extreme impairment of pulmonary diffusion are predominantly those of a large right-to-left shunt. A revised approach is suggested for the calculation of venous admixture when the latter results from extreme diffusion impairment. The approach is based on taking the entire A-a gradient and calling it venous admixture (after authors' summary).—C. H. Pierce

Pulmonary Function Studies in Children. H. A. Lyons*, R. W. Tanner, and T. Picco. *AMA J Dis Child*, August, 1960, 100: 196–207. (*450 Clarkson Ave., Brooklyn, N.Y.)

Determinations of vital capacity (VC), maximal expiratory flow rate (MEFR), and timed vital capacity (TVC) were made for 1,163 healthy school children. The mean values, range of values, and standard deviations were tabulated for the age groups from 6 to 14 years. Comparisons of these values were made with previously published values. Prediction formulas were derived for VC and MEFR and were statistically evaluated. Values less than 75 per cent of the predicted VC and less than 70 per cent of the predicted MEFR are considered subnormal for either sex. Nomograms were constructed from the prediction formulas in order to simplify the prediction of pediatric VC and MEFR (after authors' summary).—M. J. Small

Pulmonary Function Studies in Older Patients with Pulmonary Tuberculosis. An Analysis of the Single-Breath Nitrogen Test (in Japanese). Y. Kasuga*. *Clin Resp Organs*, August, 1960, 15: 551–555. (*National Kanagawa Sanatorium, Hatano, Japan)

Because of the increasing number of such patients in recent years, pulmonary function was studied in 45 patients of at least 50 years of age with pulmonary tuberculosis. Patients in this age group had ventilatory insufficiency of the restrictive type and uneven distribution of inspired air. The author performed the single-breath nitrogen test according to Fowler's method. After a single maximal inhalation of 100 per cent oxygen, the concentration of nitrogen in the expired gas was recorded constantly, and the difference in the nitrogen concentrations when the patient expired 750 ml. and 1,250 ml. of alveolar gas was obtained and called the ΔN_{250}. It was normal in young healthy subjects, but was either normal or abnormal in older healthy subjects. This procedure was therefore considered to be useless as a screening test of the ventilatory function in the older age group subjects. Following 7 minutes of inhalation of 100 per cent oxygen, the concentration of nitrogen in the expired air, both in the resting state and after forceful expiration, was obtained, and the difference was designated as the ΔN_2. This value and the intrapulmonary gas mixing index correlated with age and showed that older people tended to develop disturbances in distribution of inspired gas.—I. Tateno

Simplified Pulmonary Physiology for Office Practice. R. Stonehill* and J. Reed. *Ann Intern Med*, July, 1960, 53: 106–115. (*USAF Hosp., Lackland, Texas)

Information necessary for an accurate clinical evaluation of ventilatory function can be obtained by using only the Warring-Siemsen Ventube and the McKesson-Scott vitalometer. These instruments are inexpensive, their combined published list price being $125.00. They are small enough to fit into a desk drawer. They can be accurately used by unskilled personnel after only moments of instruction and practice. Sterilization requires only immersion of the mouthpiece in alcohol. Maintenance rarely extends beyond cleaning. The entire test series can be accomplished in approximately 15 minutes. On 200 consecutive referrals (representing 177 patients) to the Pulmonary Physiology Laboratory, each patient was tested both by the standard methods and by this simplified investigative procedure. What is its clinical significance in relation to dyspnea? With the Warring-Siemsen Ventube and McKesson-Scott vitalometer, it was possible to demonstrate a high degree of clinical accuracy and to evaluate objectively the functional progress of the disease and the response to therapy.—T. H. Noehren

Studies of the Mechanics of Breathing in Children with Congenital Heart Lesions. G. Wallgren*, F. Geubelle, and G. Koch. *Acta Paediat (Upps)*, July, 1960, 49: 415–425. (*Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden)

As a prelude to cardiac catheterization, 25 children, aged 1 day to 15 years, who had various congenital cardiac conditions were investigated with respect to their pulmonary compliance. It was found that the premedication routinely given to these children prior to catheterization sedated them sufficiently to facilitate the investigation (even in children less than 7 years of age) without apparently interfering with the determined values of lung compliance. Normal newborns and children admitted to the hospital for minor surgery served as the control group and were studied under conditions identical to those employed for

the cardiac group. The cardiac group had a significantly lower compliance than the control group. In agreement with the findings in older children and adults, no correlation was found between the reduction in compliance and the pressure in the pulmonary artery, the left atrium, or in the pulmonary capillary wedge, nor in the amount of flow in the pulmonary vascular bed. The authors conclude that rather than being directly due to hemodynamic alterations in the lungs, this reduction in compliance is probably due to a decreased pulmonary capacity. Roentgenographic studies suggest that the space-occupying influence of the enlarged heart may have a significant bearing on the compliance of the lungs in these children. Further studies in regard to this relationship are in progress (after authors' summary).—H. J. Simon

Ventilatory Insufficiency in Extreme Obesity. J. Pedersen* and E. Torp-Pedersen. *Acta Med Scand*, August 10, 1960, 167: 343–352. (*Bispebjerg Hosp., Copenhagen, Denmark)

A case of extreme obesity is reported in a male weighing 105 kg. who had a typical ventilatory insufficiency syndrome involving hypoxia, hypercapnia, cyanosis, polycythemia, and somnolence. After weight reduction (25 kg.) the symptoms lessened, and the patient was again able to work. The following parameters were normal or around the lower limit of normal, and did not change during the weight reduction: total, vital, and residual capacity as well as residual volume, respiratory rate, compliance, resistance, and Tiffeneau (one-second vital capacity) values. On the other hand, the tidal volume and minute ventilation rose, as did, although less markedly, the expiratory reserve volume. Only the last-mentioned value had been slightly reduced prior to weight reduction. Moreover, there was a rise in the carbon dioxide sensitivity of the respiratory center which had been greatly reduced before the weight reduction. The tidal volume showed a close positive correlation with the weight loss. These was also a close positive and negative correlation, respectively, between the tidal volume and the arterial oxygen saturation and carbon dioxide tension during the weight reduction. The most likely explanation of the syndrome is assumed to be that alveolar hypoventilation arises when the increased work of breathing due to fatty infiltration of the chest bellows exceeds a critical limit.—E. Dunner

Effect of 14-Hydroxydihydromorphinone on Respiration. M. E. Resnick*, R. D. Berkowitz, T. Rodman, and H. P. Close. *JAMA*, August 13, 1960, 173: 1649–1653. (*Veterans Hosp., Philadelphia, Penn.)

The effect of a new synthetic morphine derivative, oxymorphone (Numorphan®), on the respiratory center was studied in 6 healthy and 10 chronically ill individuals. The administration of 1.5 mg. subcutaneously resulted in respiratory depression in all 16 persons and was most marked in older people.—H. Abeles

Respiratory Functions after Pneumonectomy. J. Mathur* and P. Bahadur. *Indian J Tuberc*, June, 1960, 7: 94–103. (*King George's Med. College, Lucknow, India)

Detailed pulmonary function studies were carried out 1 week preoperatively and 2 months postoperatively in 10 patients. In none of the cases was a thoracoplasty performed. No evidences of postoperative emphysema of the remaining lung were found. The exercise tolerance index is believed to be a reliable test for determining the degree of postoperative invalidism. There is danger of producing respiratory crippling in the patient with a preoperative breathing reserve of less than 85 per cent of the maximal breathing capacity and an exercise tolerance index of less than 77.
—E. A. Rouff

Respiratory Studies in Newborn Infants. I: Apparatus and Methods for Studies of Pulmonary Ventilation and the Mechanics of Breathing. Principles of Analysis in Mechanics of Breathing. P. Karlberg*, R. B. Cherry, F. Escardo, and G. Koch. *Acta Paediat (Upps)*, July, 1960, 49: 345–357. (*Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden)

The apparatus and methods are described for studying the pulmonary ventilation and the mechanics of breathing in newborn infants. For volume recording with long duration a body plethysmograph system is used; for short recording periods, a reverse plethysmograph system is employed. For studies of the mechanics of breathing, the intraesophageal pressure changes were recorded simultaneously with the volume changes occurring during single respiratory cycles. The reproducibility and calibration of the methods are explained. The technique has been used in respiratory studies in the neonatal period and the results will be published at a later date in this journal. An outline of the principles of analysis of the mechanics of breathing and the different methods used for calculations are also given.—H. J. Simon

The Pulmonary Vascular Bed in Tetralogy of Fallot. I. Changes Associated with Pulmonic Stenosis. II. Changes Following a Systemic-Pulmonary Arterial Anastomosis. C. Ferencz*. *Bull Johns Hopkins Hosp*, No. 2, 1960, 106: 81–118. (*Johns Hopkins U. School of Med., Baltimore, Md.)

These studies were undertaken to obtain further information on the anatomic state of pulmonary vessels in patients with the tetralogy of Fallot, and to observe the possible alterations following a systemic-pulmonary anastomosis; 145 patients who were followed since 1945 and upon whom autopsies had been performed comprise the subject of this report. The findings from microscopic examinations of lung sections after hematoxylin and eosin staining and duplicate sections processed with Verhoeff's elastic tissue stain were correlated with the clinical data. The results are discussed in detail, and a large series of photomicrographs serve to document the observations. Severe disturbance of the pulmonary circulation was evident in these patients. The creation of a systemic-pulmonary anastomosis caused either detrimental or beneficial alterations in the pulmonary vascular bed.—C. H. Pierce

MICROBIOLOGY AND IMMUNOLOGY

Extracellular Acids Produced by *Mycobacterium Ranae* and *Mycobacterium Tuberculosis* H37Rv. A. V. Fowler*, M. N. Camien, and M. S. Dunn. *J Biol Chem*, May, 1960, 235: 1386–1389. (*U. of California, Los Angeles)

Three acids were isolated in crystalline form from culture filtrates of *M. ranae* and *M. tuberculosis* H37Rv. These acids were identified as succinic acid, fumaric acid, and acetic acid. A fourth acid isolated in crystalline form from *M. ranae* was identified as DL-5-carboxy-methylhydantoin.
—E. S. Leake

A Study of *Cryptococcus Neoformans* by Means of the Fluorescent Antibody Technic. A. Kase* and J. D. Marshall, Jr. *Amer J Clin Path*, July, 1960, 34: 52–56. (*Armed Forces Institute of Pathology, Washington, D. C.)

The fluorescent antibody method has been studied for use in identifying *Cryptococcus neoformans*. Of 96 strains of *C. neoformans* and *C. neoformans* var. *innocuous* tested, 95 reacted regardless of the amount of capsule present. Fixation by various means did not affect the staining reaction.
—E. Dunner

A Method for Studying Micro-colonial Structure and Cord Formation. J. Lloyd*. *Tubercle*, August, 1960, 41: 281. (*Postgraduate Med. School of London, England)

Microcolonial structure and cord formation are of value in the systematic study of mycobacteria and nocardiae. Microcolonies can be stained undisturbed when grown on membrane filters, but a simpler method is described here. With tubercle bacilli, cord formation at an early stage was seen after 5 days incubation, but it was well marked after 7 days. The microcolonial morphology of strains of nocardiae remained undisturbed during growth and staining. Even after 14 days the drops of Kirschner medium had not evaporated.
—M. J. Small

The So-called Atypical (Unclassified) Mycobacteria (in German). E. Freerksen*, *Klin Wschr*, April, 1960, 38: 297–309. (*Tuberkuloseforschungsinstitut Borstel, Germany)

This is an historic review dealing with all mycobacteria classified as "atypical" (unclassified). An important property of these strains is the lack of virulence for guinea pigs. Most of the strains can be included in the 4 groups classified by Runyon; the new biochemical tests may allow further differentiation. All of these mycobacteria are pathogenic; some are also potentially virulent for man—a suggested distinction between pathogenicity and virulence. Several of the strains possess some immunizing potency against virulent superinfection; this faculty, however, is less than that of BCG. In animal experiments, BCG is effective against superinfection by unclassified strains. The unclassified mycobacteria can be found in nature, as opposed to human, bovine, and avian tubercle bacilli; this fact presents an ever-present possibility of infection in man and animal. Infection of man by unclassified mycobacteria leads to a positive tuberculin reaction in most cases; in animal experiments, however, the tuberculin reaction is not always positive. The term "atypical" is misleading since it assumes the existence of a "typical" species of mycobacteria. But it has never been made clear which strain of these bacilli is "typical" and on what basis it is judged so.—Z. Virágh

Biochemical Characteristics of Atypical (Unclassified) Acid-fast Bacilli (Atypical Chromogenic Mycobacteria) (in Japanese). M. Tsukamura*. *Kekkaku*, August, 1960, 35: 576–580. (*National Obuso Sanatorium, Aichi Prefecture, Japan)

The biochemical characteristics of 8 strains of scotochromogenic "unclassified chromogenic mycobacteria" were subjected to study. The cultures used in the experiment were in the early stage of the stationary growth phase on Ogawa medium. Four of the 8 strains had a high ribonucleic acid: desoxyribonucleic acid (RNA:DNA) ratio of more than 15, while it was usually less than 10 in the case of known mycobacteria (*M. tuberculosis*, *M. smegmatis*, and *M. avium*). Six of 8 strains had a high picric acid–reducing activity in the pres-

ence of glycerol which was not observed in the case of known mycobacteria. The writer concludes that by determining the DNA content, the ratio of the contents of RNA and DNA, and the picric acid-reducing property without substrate and in the presence of various substrates of unclassified chromogenic mycobacteria, they may be classified into 2 subgroups.—I. Tateno

The Neutral Red Test. A Method of Determining Virulence of Mycobacteria. M. B. Jefferies*, N. J. Schneider, and A. V. Hardy. *US Armed Forces Med J*, August, 1960, 11: 927-931. (*Florida State Board of Health, Jacksonville)

A study has been conducted in this laboratory since 1956 to compare the neutral-red test and guinea pig method for the determination of virulence of acid-fast bacilli. A detailed description is given of the techniques employed. Of 253 cultures of acid-fast organisms examined, 175 strains culturally determined as *M. tuberculosis* were neutral-red-positive and produced characteristic disease in guinea pigs. Negative findings were obtained with both tests in the case of 30 strains culturally resembling acid-fast saprophytes. Forty-eight strains were neutral-red-positive, but did not produce lesions beyond the site of inoculation in guinea pigs. Of this latter group, 10 strains were from tuberculosis patients undergoing antimicrobial therapy, and 15 were obtained from patients known to be discharging unclassified acid-fast bacilli. No information was available to explain the results of the remaining 23 cultures. The findings in this study suggest that the neutral-red test would be useful under certain conditions in a routine diagnostic laboratory as an adjunct procedure for determining virulence of *M. tuberculosis*. To rely on the neutral-red reaction without taking into account the susceptibility of organisms to isoniazid would be misleading. Furthermore, the neutral-red test does not distinguish between virulent strains of *M. tuberculosis* and neutral-red-positive strains of unclassified mycobacteria, many of the latter having little or no virulence for man.
—C. H. Pierce

Enumeration of the Viable Number of Tubercle Bacilli by the Utilization of Spiral Loop Inoculation and Egg Medium Slants. III. (in Japanese). M. Tsukamura*. *Kekkaku*, June, 1960, 35: 397-399. (*Obuso Sanatorium, Aichi Prefecture, Japan)

The writer devised a simple method of counting the viable number of tubercle bacilli in the sputum by inoculating, quantitatively, 0.02 ml. of alkali-digested material by spiral loop onto egg medium slant ("1 per cent Ogawa's medium"), instead of inoculating 0.1 ml. to "3 per cent Ogawa's medium," as currently done in Japan. The new method allows the inoculated tubes to be immediately rubber-stoppered and kept upright, whereas the former method required the inoculated tubes to be kept overnight horizontally for drying, and then rubber-stoppered and incubated. There was no significant difference between the rates of positive cultures and between the coefficients of variation occurring in estimating the number of viable cells, although the old method had a tendency to give a somewhat higher rate of positive cultures, and larger errors of estimation. It appeared that the number of viable cells counted by the old method was less than the actual number of cells present in the test material, as the number of cells grown by the old method (0.1 ml.) was not more than twice that obtained by the new method (0.02 ml.).
—I. Tateno

Electrophoretic Concentration of Tubercle Bacilli in the Sputum and Urine (in Japanese). N. Kusunoki* and F. Kumada. *Kekkaku*, August, 1960, 35: 567-569. (*Fukushima Med. College, Japan)

In view of the increasing frequency of the observance of culture-negative smear-positive tubercle bacilli in recent years, re-evaluation of the method of concentrating tubercle bacilli in the sputum and urine, et cetera, appeared worth while. The writers treated 1 to 3 ml. of sputum with 1 to 3 drops of 8 per cent NaOH, and then added 10 to 20 volumes of distilled water. The homogenate was centrifuged at 3,000 r.p.m. for 20 minutes and the sediment was smeared on a slide over an area of 1.5 $cm.^2$. An electric potential was then applied to both ends of a diagonal of the square through lead wires from a 6-volt dry battery. After 30 minutes the electric current was stopped and the smear was stained by Ziehl's method. The tubercle bacilli were found almost exclusively in a few fields of anode extremity: 112 specimens of the sputum which were negative for tubercle bacilli by ordinary smear examination were subjected to comparative study; 43 specimens were positive by this electrophoretic concentration method, 16 were positive by culture, and 26 were positive by the ordinary concentration method. Only 2 specimens were positive by culture and negative by electrophoretic concentration, while 29 specimens were culture-negative and positive by electrophoretic concentration.—I. Tateno

EXPERIMENTAL PATHOLOGY

Histology of Chronic Bronchitis (in Japanese). A. Yamanaka*. *Saishin Igaku*, August, 1960, 15: 2035-2044. (*St. Luke's International Hosp., Tokyo, Japan)

The writer made a review and analysis of the histology of chronic bronchitis and stressed the importance of peribronchitis and peribronchiolitis in the establishment and clinical course of chronic bronchitis. In order to further substantiate his opinion, he presented detailed clinical and histologic reports on 2 cases each of chronic atrophic bronchitis, diffuse chronic bronchiolitis with cor pulmonale, and chronic ulcerative (proliferative) bronchitis associated with cor pulmonale. He concludes by saying that this is only the initial step in the study of the pathology of chronic bronchitis, and many untouched aspects of the disease should be solved in future studies.—I. Tateno

The Role of Roasted Chromite Ore in the Production of Cancer. W. Payne*. *Arch Environ Health*, July, 1960, 1: 20–26. (*National Inst. of Health, Bethesda, Md.)

The results of this experiment give additional evidence that the intermediate products and the discarded residue in the production of chromium chemicals may be hazardous.—T. H. Noehren

Effect of Cortisone on Mice Infected with Chromogens and with Isoniazid Resistant Tubercle Bacilli. F. K. Fitzpatrick*. *Proc Soc Exp Biol Med*, August-September, 1960, 104: 558–560. (*Merck Inst. for Therap. Res., Rahway, N. J.)

Laboratory strains of *M. tuberculosis* H37Rv that had become resistant to isoniazid showed an increase in their virulence for mice when cortisone was administered subcutaneously. Cortisone also increased the pathogenicity of some scotochromogens and some photochromogens for mice. Seven Battey strains were unaffected.—E. S. Leake

Specific and Non-Specific Immunity in Experimental Cryptococcosis in Mice. D. B. Louria*. *J Exp Med*, No. 5, 1960, 111: 643–665. (*Second Med. Division, Bellevue Hosp., New York)

A comprehensive general report of the subject is documented by an extensive bibliography. Results obtained in the present study from detailed experimental investigations are summarized as follows: The course of lethal cryptococcosis in mice was modified by immunization with the same strain or different strains of *C. neoformans*. Protection was associated with a definite decrease in tissue fungus multiplication over the initial 7 days of infection. Such immunity was species-specific and did not protect against heterologous challenge with *Staph. aureus*, *K. pneumoniae*, or *M. tuberculosis*. Similar modification of lethal cryptococcosis was produced by intraperitoneal inoculation of 100 to 200γ of Salmonella endotoxin 7 days prior to challenge. In nonspecifically- (endotoxin) immunized mice there was only a minimal reduction in tissue cryptococci census during the first week of infection. When small numbers of virulent cryptococci were injected intravenously into normal mice, tissue populations rose markedly in all organs studied during the first 7 days. A progressive fall in tissue fungus census was then observed in most mice during the next 6 months. The increase in resistance to cryptococcal challenge following specific immunisation, the nonspecific protection conferred by injection of bacterial endotoxin, and the ability to control and eventually reduce infections with small inocula may well be dependent in each case on the development of specific antibody.—C. H. Pierce

Fever from Pathogenic Fungi. A. I. Braude*, J. McConnell, and H. Douglas. *J Clin Invest*, August, 1960, 39: 1266–1276. (*U. of Pittsburgh, Penn.)

The mechanism of fever in fungous infections was studied by systematically examining pathogenic fungi for their capacity to induce fever in rabbits. Intravenous injections of viable *Candida albicans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Sporotrichum schenckii* produced fever, but cultures of *Cryptococcus neoformans* did not. The fever from these live cultures took 2 forms, depending upon the dose and the species of fungus: (*1*) an immediate fever resembling that produced by gram-negative bacterial lipopolysaccharides, and (*2*) a delayed fever. The immediate type of fever curve was attributed to a heat-stable filterable fungus pyrogen. The delayed type of fever curve was believed to be a consequence of infection of the tissues. It was observed with experimental candidiasis and sporotrichosis and was not accompanied by positive blood cultures of the infecting fungus. For this reason, and because a circulating pyrogen was found on the second day, it is believed that either the fungus pyrogen or an endogenous tissue pyrogen is released from the infected tissue. The absence of fever after injection of cryptococci coincides with the clinical observation that this fungous disease alone is often characterized by little or no fever in the course of severe systemic human infections.—E. Dunner

The Experimental Disease that Follows the Injection of Human Leprosy Bacilli into Foot-Pads of Mice. C. C. Shepard*. *J Exp Med*, No. 3, 1960, 112: 445–454. (*Communicable Disease Center, Montgomery, Ala.)

Specimens were obtained from lepromatous patients at Carville, Louisiana, and Tala, the Philippines. The nasal washings, collected in balanced

salt solution to which bovine albumin was added before shipment under wet-ice refrigeration, were processed at the Montgomery laboratory. After decontamination with NaOH and concentration by centrifugation, aliquots of the sediment were examined by smear and inoculated into the hindfoot pad of CFW mice. Detailed studies were made of the progression of disease in the foot, and mouse passages were performed by foot pad inoculation with pooled suspensions of harvested feet from 4 infected mice. The original materials for mouse injection, as well as those obtained from harvesting, were inoculated into the following bacteriologic media: Löwenstein-Jensen, 25 per cent blood agar, and 7H9 agar with oleic acid–albumin supplement. Most of the same materials were also inoculated into cultures of HeLa cells. Skin biopsies collected at Carville were shipped without the addition of fluid, and treated according to a similar procedure. Microscopic granuloma containing acid-fast bacilli developed in the foot pads of mice inoculated with nasal washings positive for leprosy bacilli in 22 of 22 instances, in 12 of 16 instances with material from positive skin biopsies, and in none of 16 cases with negative nasal washings. The incubation period in the mouse was 1 to 2 months with a dose of 10^6 bacil'i, and 6 months with 10^5 organisms. After development of the lesion, the number of bacilli harvested was about 10^6, regardless of the number inoculated. Passage to new groups of mice was successful 11 of 12 times. One strain has been through 4 passages. Growth of mycobacteria was not obtained on the media employed.
—C. H. Pierce

The Effect of Macrocyclon on the Multiplication of Tubercle Bacilli in the Lungs and Spleen of Mice. J. Niffenegger* and G. P. Youmans. *Brit J Exp Path*, August, 1960, 41: 403–413. (*Northwestern U. Med. School, Chicago, Ill.)

Male and female mice of the "Strong A" strain were infected intravenously with *M. tuberculosis* H37Rv. Some of the infected mice had received 25 mg. of macrocyclon intraperitoneally 1 or 28 days prior to infection, some had received 1.0 mg. of freeze-dried BCG intraperitoneally 28 days previously, and others were untreated. At weekly intervals following infection, 5 mice from each group were killed. The lungs, and in some cases the spleens, were removed aseptically, weighed, and homogenized. Serial dilutions of the tissues were planted on Proskauer and Beck medium. At the end of 21 days, the number of colonies was counted. The results showed that macrocyclon as well as BCG checked the multiplication of tubercle bacilli in the organs of mice.—H. J. Henderson

Effect of Macrocyclon in Acute and Chronic Pulmonary Tuberculous Infection in Mice as Shown by Viable and Total Bacterial Counts. P. D'Arcy Hart* and R. J. W. Rees. *Brit J Exp Path*, August, 1960, 41: 414–421. (*Nat. Inst. Med. Research, Mill Hill, London)

The effect of macrocyclon, a surface-active agent that is antituberculous *in vivo* but not *in vitro*, was studied in tuberculous mice by dual bacterial counts—viable and total (stainable)—in lung homogenates. Two types of experimental infection were used: acute and fatal produced by a large dose of bacilli, and chronic, following a smaller dose. The drug was given subcutaneously twice weekly, from 2 until 26 days after the acute infection and from 60 until 131 days after the chronic infection. In the acute infection the total counts from the lungs of untreated mice were very similar to the viable counts throughout, indicating that most of the bacterial population was viable; and the continuous rise in both counts showed that the population was multiplying. The counts from the treated mice diverged from these counts, indicating a definite though not powerful antibacterial effect which was more manifest after the first week and even more so after a fortnight. From the close similarity of the total and viable counts in the treated group it was concluded that macrocyclon's action was bacteriostatic rather than bactericidal. In the chronic infection the two counts from the untreated mice were also very similar, indicating that here too most of the bacterial population was viable, without a build-up of dead bacilli in the lungs; but (unlike the acute infection) both counts remained constant throughout the observation period, indicating a steady population. The trend of the counts from the treated mice showed no evidence of any antibacterial effect by macrocyclon. It was considered that, unless dead organisms were being removed from the lungs of the untreated animals, the majority of the bacilli were not multiplying and that in this resting phase they may not have been susceptible to the drug's action (Authors' summary).—H. J. Henderson

Organ Cultures of Human Fetal Skin for the Study of Skin Diseases. T. R. McGowan* and F. B. Bang. *Bull Johns Hopkins Hosp*, No. 2, 1960, 107: 63–71. (*Johns Hopkins U. School of Hyg. & Public Health, Baltimore, Md.)

Organ cultures of full-thickness human skin have been maintained independently of circulation, and in circumstances under which various skin diseases can be studied. The optimal source was from fetuses 13 to 26 cm. in length. Explants of such skin remained essentially constant in size but showed internal development and differentia-

tion. Optimal temperatures of incubation were 33°C. to 37°C., and optimal nutrition was provided by a clot which consisted of 12 per cent chick embryo extract and 12 per cent saline in 76 per cent chicken plasma. Under these conditions 90 per cent of the explants remained viable and useful for periods up to 6 months. Many of the changes which develop in intact normal and infected skin could be studied in serial sections stained with hematoxylin and eosin. Inoculation with herpes simplex virus caused characteristic cytologic and histologic changes. It is suggested that such tissues offer a favorable medium for the study of a variety of skin diseases (after authors' summary).—C. H. Pierce

Studies in Tuberculo-Immunity. H. S. Willis*, H. M. Vandiviere, M. R. Vandiviere, and I. Melvin. *Amer J Med Sci*, August, 1960, 240: 137–158. (*N. Carolina Sanatorium System, Chapel Hill)

Study of the bovine culture BCG demonstrates that there is not one BCG strain but several strains with widely varying potency and divergent capacity to produce immunity. These various strains of BCG were compared with the attenuated human strain R1 by: (*1*) using such criteria of potency as cord-formation titer and the omental index; (*2*) measuring the duration and volume of allergic response after animal inoculation; and (*3*) determining the degree and duration of protection conferred against challenge. Differences were marked. The ascending scale of biologic response, BCG No. 1 to R1, as shown in the duration and extent of tuberculin reactions in inoculated animals, corresponded to the ascending scale of cord formation and of omental index. The protection afforded animals by inoculation varies with the potency of the strain used, its dose, and the duration of inoculation period prior to challenge, as well as with the dose of the challenge infection. A very high degree of protection may be afforded by use of proper strain and dose and proper duration of time between vaccination and challenge. In these experiments with approximately 7,000 animals, the highest degree of immunologic protection resulted from vaccination with the attenuated human strain R1. Quite close to this were BCG No. 3 and No. 4. Other BCG strains studied fell far below this degree of protection and exerted little or no effect upon the course of infection following challenge.—W. J. Steininger

Development of Tuberculous Allergy after Inoculation with BCG Vaccine in Offspring of Healthy and Tuberculous Female Guinea Pigs: Investigations with BCG Tuberculin and Old Tuberculin. A. Lithander*. *Acta Path Microbiol Scand*, Fasc. 2, 1960, 49: 165–175. (*Serafimerlasarettet, Stockholm, Sweden)

Guinea pigs vaccinated with BCG vaccine at an early age have little tendency to produce allergy. Their capacity to produce allergy rises with increasing age at vaccination. In the offspring of healthy females, the frequent presence of allergic animals shows a sharp rise when the age at vaccination is increased from 6 to 8 weeks, which is probably due to an accelerated immunologic maturation within this age period. In the offspring of tuberculous females, this phenomenon does not appear. After BCG vaccination, in tests with both BCG and OT, the frequency of positive reactions in the offspring of healthy females is higher than in those of tuberculous females. The differences in allergy between the offspring of healthy and those of tuberculous females are due to an interference by a factor transferred from the tuberculous mother. This interference is mainly apparent in tests with OT, and decreases with age at vaccination. The lower frequency of positive reactions to BCG in the offspring of tuberculous females is due to a partial interference. The frequency of positive reactions with BCG was higher than that with OT in the offspring of both healthy and tuberculous females, and at all ages of vaccination. The findings in guinea pigs that, after BCG vaccination, the offspring of tuberculous females become positive to tuberculin later than those of healthy females, are in agreement with observations made in investigations on children of tuberculous and healthy mothers. A postponement of the BCG vaccination until after the age of 2 to 3 months is suggested (after author's summary). —E. Dunner

PUBLIC HEALTH AND EPIDEMIOLOGY

Multiple Puncture BCG Vaccination Using 35 Months Old Freeze-Dried Vaccine. A. H. Griffith*. *Tubercle*, June, 1960, 41: 178–180. (*Health Dept., City Hall, Cardiff, Wales)

A total of 55 tuberculin-negative 13- and 14-year-old children were BCG vaccinated by means of the multiple-puncture technique using freeze-dried vaccine that was 35 months old. The multiple-puncture technique employed is described. All of the children were found to be tuberculin positive after 95 days, and the BCG did not produce ulcerative lesions in a single instance (after author's summary).—M. J. Small

Effect of Repeated Fluoroscopic Examinations on 1480 Children With a Long-Term Follow-up Study. A. M. Birch* and D. H. Baker. *New*

Engl J Med, May 19, 1960, 262: 1004–1008. (*Cornell U. Med. College, N.Y., N.Y.)

The charts were reviewed of 1,480 patients who had repeated fluoroscopic examinations and were followed closely. Two cases of cancer developed during 26,314 patient-years of observation. This was found to be below the expected incidence in the general population according to age and sex. There was no statistically significant difference in the stillbirth rate compared with that of the general population, either in the over-all group of 1,087 births or in the 121 pregnancies during which fluoroscopy was performed. The rate of congenital anomalies (2.1 per cent) was similar to that reported from the literature for the general population. The absence of observed deleterious effects in a group of this size indicates that the risk to the individual patient was too low to be observed in a population of this size.—M. J. Small

Neoplasia Following Therapeutic Irradiation for Benign Conditions in Childhood. E. L. Saenger*, F. N. Silverman, T. D. Sterling, and M. E. Turner. *Radiology*, June, 1960, 74: 889–904. (*Cincinnati General Hosp., Ohio)

A series of 1,644 persons of a total of 2,230 who in infancy and childhood had irradiation to the head, neck, and chest were compared to 3,777 siblings with respect to subsequent medical history. Eleven carcinomas of the thyroid were found in the patient group and none in the sibling controls. This incidence in the irradiated group is about 100 times that expected from current tables of morbidity and is comparable to that reported in other similar surveys. The incidence of infections and other non-neoplastic illness was greater among the patients than among the siblings. However, a statistically significant difference in the mortality ratio in the 2 groups was also found: the death rate in the siblings was greater than that in the patients. Although there is a significant increase in the incidence of cancer of the thyroid following irradiation, radiation does not appear to be the sole factor responsible for the increased incidence but, rather, a contributing factor. This survey differs from previously reported surveys in the extent to which the total family health history was evaluated. The information obtained is useful in placing in perspective possible carcinogenic factors other than radiation.—W. J. Steininger

A Simple Test for PAS in Urine. S. Frostad*. *Tubercle*, June, 1960, 41: 221–223. (*Grefsen Tuberculosis Hosp., Oslo, Norway)

A simple and inexpensive method of detecting PAS in urine is described, using strips of filter paper soaked in 3 per cent ferric chloride solution. Thin, unprepared sheets of filter paper are soaked in a 3 per cent solution of ferric chloride in flat-bottomed dishes to ensure even distribution. The paper, now a light yellow color, is dried and cut into small strips. One of the strips is dipped into the undiluted urine. The presence of PAS is indicated by a change from yellow to various shades of violet-blue. Low concentrations give a light blue color and high ones a brownish-blue. The ferric chloride test is not specific for PAS, the reaction being given by other salicylates. The advantages of the method here described are its cheapness and its great simplicity.—M. J. Small

New Stick Test for P.A.S. in Urine. G. R. W. N. Luntz* and R. Austin. *Brit Med J*, June 4, 1960, No. 5187: 1679–1684. (*Birmingham Chest Clinic, England)

A new test for PAS in urine (Phenistix reagent strips) is described. Phenistix has a good degree of specificity for PAS, and no essential difference was noted whether the sodium or the calcium salt of PAS had been taken. Positive results for reasons other than PAS were relatively few; 1,370 consecutive new outpatients were examined and positive results were obtained with Phenistix in 91 (7 per cent), the majority due to acetylsalicylic acid. The extreme simplicity of Phenistix, together with its optimal sensitivity, makes it the ideal test for the qualitative estimation of PAS in urine in both outpatients and inpatients. A negative Phenistix test is evidence that no appreciable amount of PAS is present in the urine and that no PAS has been taken for 12 to 18 hours. Of a total of 444 tests at unexpected times in hospital inpatients receiving PAS, 436 (98 per cent) gave positive and 8 (2 per cent) negative results. Not all patients in hospital can be relied on to take their medicine unless the nurse makes sure that the medicine is actually swallowed. Of 705 tuberculous outpatients on PAS therapy, 467 gave positive Phenistix results, and the remaining 238 (34 per cent) were "defaulters." Negative results were found in 39 per cent of the women and 30 per cent of the men. The length of time that the patients had been taking chemotherapy and the time elapsing since discharge from hospital had a significant effect on the results. The failure rate increased steadily in both instances with each year of duration of chemotherapy. It is inadvisable to permit young adults aged 15–24 of either sex, females aged 25–44, and males older than 55 to leave hospital after only a short stay, as patients in these age groups showed the highest default rates. Self-testing in 20 patients revealed the reasons for negative tests and gave insight into the patients' drug-taking habits. The problem of self-administration of drugs is discussed. It is suggested that

the best results will be obtained in cases in which the whole course of treatment is directed by one experienced physician, and that continuity of medical surveillance is essential (after authors' summary).—E. A. RILEY

Morphogenesis of Pneumoconiosis Occurring in Southern Illinois Bituminous Workers. J. WYATT* and V. FISCHER. *AMA Arch Industr Health*, May, 1960, 21: 445–457. (*St. Louis U., Missouri)

Six cases have been selected to establish the existence and structural pattern of the pneumoconiosis observed in southern Illinois coal fields. Comparison of these anatomic patterns with the focal emphysema observed in the South Wales coal workers and the generalized focal emphysema in nonindustrial groups establishes a basic pathogenetic mechanism accountable for the koniotic emphysema in southern Illinois coal miners. Exposure to coal in southern Illinois coal fields is capable of producing a coal macule–emphysema pneumoconiosis akin to that observed in South Wales but, in this study, the incidence and anatomic extent of the generalized dust lesion appeared to be reduced in frequency and severity. The reactive response of emphysema in the older workers, probably initiated by bronchiolar dilatation, often outstrips the amount of coaly deposits in the lung, suggesting that other factors may be responsible for the progress and development of emphysema into a form with bullous fields and large expanses of "pathological dead space." Desooting of the lung may occur after exposure to coal has stopped, and leaves a structural pattern of deranged lung as an aftermath which may sometimes be difficult to relate to an occupational hazard. Because of the varying mineralogic strata in coal fields throughout the United States, an analysis of coal workers' lungs from these different coal fields utilizing the macromap technique is important. The whole-lung parchment presents a superior method of correlating pathologic lesions with clinical findings, and is a reliable and permanent medicolegal "document" offering orientation of physical and chemical findings on the anatomic stratum.—T. H. NOEHREN

Pulmonary Siderosis in Electrical Welder. L. ANGERVALL*, G. HANSSON, and H. RÖCKERT. *Acta Path Microbiol Scand*, Fasc. 3, 1960, 49: 373–380. (*U. of Gothenburg, Sweden)

Autopsy findings are reported in a case of pulmonary siderosis in an electrical welder who died of lobar pneumonia at the age of 57. The markedly anthracotic lungs contained copious amounts of iron particles predominantly located perivascularly, intra-alveolarly, in the alveolar septa, and subpleurally, as well as within the regional lymph nodes. Chemical analysis showed that the pulmonary tissue contained normal amounts of silicon dioxide, and excessive amounts of iron oxide. Microroentgenograms displayed excellently the location in the pulmonary tissue of the iron particles. Fairly mild fibrosis was present at the perivascular and subpleural sites of accumulations of iron particles, and it is postulated that iron oxide, at least in large amounts, is capable of inducing some degree of reactive fibrosis.
—E. DUNNER

Silicosis in Nonmining Industries (in Spanish). V. MATURANA*, E. CRUZ, P. SCHÜLER, C. GUIJÓN, A. VALENZUELA, and H. OYANGUREN. *Rev Chil Enferm Torax*, 1959, 24: 54–66. (*Servicio de Higiene y Seguridad Industrial de Santiago, Chile)

In Santiago de Chile, 5,776 workers are employed in 21 nonmining industries. The records of 276 workers with pneumoconiosis were analyzed for the period 1941–1959. The workers in quartz mills and abrasive soap factories are more apt to develop silicosis; this risk is smaller in glass, ceramic, and tile industries, and still less in the metallurgic industries and in foundry workers. Tuberculosis is a frequent associated disease, especially in the workers with the greatest exposure to silica-containing dust.—Z. VIRÁGH

Is Cigarette Smoking a Cause of Carcinoma of the Bronchus? D. T. CARR*. *Proc Mayo Clin*, June 22, 1960, 35: 358–367. (*Mayo Clinic, Rochester, Minn.)

The author gives a partial review of the literature on the relationship between smoking and the development of epidermoid and undifferentiated carcinoma of the lung, and concludes that cigarette smoking is a cause of carcinoma of the bronchus. He believes that all physicians should give this information to their patients, that appropriate health agencies should initiate an intensive educational program directed at teenagers, and that the tobacco industry should stop denying the dangers of smoking and should attempt to produce a safe cigarette.—E. A. RILEY

Smoking and Cancer of the Lung. J. BERKSON*. *Proc Mayo Clin*, June 22, 1960, 35: 367–385. (*Mayo Clinic, Rochester, Minn.)

After reviewing all of the accumulated evidence, the author concludes that it is very doubtful that smoking causes cancer of the lung. Citing the fact that the whole support for this theory comes from statistics rather than clinical, pathologic, or experimental studies, he states that the definitive finding in all prospective statistical studies is not

that there is an association between smoking and cancer of the lung, but that there is a statistical association between smoking and death from all causes generally, of which cancer of the lung is only a small part. One explanation for these findings is that the statistics are spurious and are related to the vagaries of the obtained data; the other is that nonsmokers are constitutionally disposed to longevity and, therefore, to generally low death rates, and that the disposition of these individuals not to smoke is one reflection of this constitution.—E. A. RILEY

Smoking Habits Related to Injury and Absenteeism in Industry. C. R. LOWE*. *Brit J Prev Soc Med*, April, 1960, 14: 57–63. (*U. of Birmingham, England)

The frequency was examined of attendance at ambulance rooms for treatment of injury and of absenteeism among 3,341 male factory workers in relation to their smoking habits. Frequency of attendance for treatment of injury decreased with age. It was higher among smokers than nonsmokers and increased fairly regularly with the amount smoked. The relationship to smoking was more pronounced among younger than among older workers. Absenteeism for medical reasons increased with age. It was higher among smokers than nonsmokers and increased with the amount smoked. The relationship to smoking was more pronounced among older than among younger workers. Absenteeism for nonmedical reasons did not appear to be related to age or to smoking habits. The bearing of these observations on the smoking controversy is discussed (after author's summary).—H. ABELES

Experience in Tracing Sources of Infection of Tuberculin Converters. D. VAN ZWANENBERG*. *Tubercle*, June, 1960, 41: 171–177. (*St. Helen's Hosp., Ipswich, England)

A tuberculin index was established to record on a punch-card system the results of all tuberculin tests in the area. Information was obtained from school surveys, chest clinics, contacts, and hospitals. As a result, 109 children were found to have developed tuberculin sensitivity, and 95 of these children and their households have thus far been carefully investigated. In 31 a probable source of infection has been discovered, but in 64 no source was found. More sources of infection were discovered when the interval between negative and positive tests was less than a year, when all of the children in the household were tuberculin positive, and when the child developed a manifest tuberculous lesion. As a result of this work, 7 new cases of tuberculosis were discovered; 17 of the children had been infected by cases already known to a chest clinic. It is concluded that this technique by itself is at present insufficient to control tuberculosis in a community where there is a large population and where communications are extensive.—M. J. SMALL

The Efficacy of the Tuberculin Test. J. NYBOE*. *Bull WHO*, No. 1–2, 1960, 22: 5–37. (*W. H. O., Geneva, Switzerland)

In order to study the efficacy of the tuberculin test, the results of the test in about 190,000 persons in Africa, America, Asia, and Europe were studied. The patterns of tuberculin sensitivity were quantitatively evaluated: intradermal 5- and 100-TU tests were used, the size of the reactions were measured, and the resulting distributions of reactions according to size were subjected to statistical analysis. The pattern of tuberculin sensitivity varies widely between different populations, but follows a definite geographic trend. In temperate and subtropical countries, almost all test reactions are either clearly positive or clearly negative, indicating that the test is highly efficient. In tropical regions, a large proportion of the reactions are intermediate in size, and distinction between the two types of clear-cut reactions is therefore difficult. The data strongly suggest that the cause of the intermediate reactions is that the population is being massively exposed to certain unidentified agents producing cross-reactions to tuberculin. In tropical regions a clear-cut distinction between tuberculosis-infected persons and the uninfected cannot be made by means of the present tuberculin test.—H. ABELES

Evaluation of the Heaf Tuberculin Test. A. B. ROBINS* and J. N. DALY. *New Engl J Med*, May 19, 1960, 262: 1008–1012. (*Bureau of Tuberc., Dept. of Health, N.Y., N.Y.)

The Heaf multiple-puncture tuberculin test was given to 61,000 pupils who entered 186 New York City high schools during 1958. In this concentrated 18-week experience it was found to be an acceptable test to both students and staff, and considerably more dependable than the Vollmer patch test. Comparative studies with the Mantoux test in a variety of groups showed the Heaf test to be highly specific, with a sensitivity level between that of intermediate- and full-strength tuberculin intradermal tests. Heaf reactions can be interpreted with a high degree of consistency, and in certain circumstances may be susceptible to reading by the subjects themselves. The Heaf test is a satisfactory substitute for the Mantoux test in mass tuberculin screening programs. There is general agreement among those who have used both methods that from the technical point of view the Heaf test is more acceptable than the

Mantoux to those giving as well as those receiving the tests. Heaf testing requires less equipment and fewer trained personnel, can be done more quickly, and offers greater flexibility in scheduling.
—M. J. SMALL

Tuberculin Testing in Ontario Mental Hospitals. A. RICE*. *Dis Chest*, June, 1960, 6: 627–631. (*Ontario Hosp., Woodstock, Canada)

Since 1937, the method used to protect the chest health of the staff in Ontario hospitals has been to obtain a chest roentgenogram and give a tuberculin test (Mantoux) to everyone entering the service. All reactors have been re-filmed annually, and all nonreactors have had tuberculin tests repeated twice yearly. Tuberculin converters are re-filmed every 3 months for 2 years, and then annually. Recently, it has been decided to make roentgenographic examinations of all staff personnel at every second annual chest survey. In addition, whenever an active case of tuberculosis was discovered in patient surveys, all staff members and patients in contact were filmed every 3 months for 1 year, and twice yearly the second year, in addition to semi-annual tuberculin tests on nonreactors. The staff now totals 7,000, with some 4,500 tests being done annually. Reactors are declining steadily, from 74 to about 66 per cent. About 33 per 1,000 converted during the 15 years of this study, men more frequently than women. About 31 per 1,000 converters progressed to active tuberculosis, approximately equally for men and women. The majority of converters becoming active did so within 4 or 5 months.
—E. A. ROUFF

The Distribution of Tuberculous Infection Among Households in African Communities. S. ANDERSEN* and A. GESER. *Bull WHO*, No. 1–2, 1960, 22: 39–60. (*Nat. Tuberculosis Inst., Bangalore, India)

An analysis of data collected in a number of territories in Africa south of the Sahara shows that, although a statistically significant accumulation of infection does occur in certain households, its extent is so moderate that it would be unjustifiable to confine control work to any limited category of households. In these areas it is necessary, therefore, to cover the entire community in antituberculosis measures designed to reduce the tuberculosis problem in the population at large.
—H. ABELES

A Community-Wide Tuberculosis Study in a South Indian Rural Population, 1950–1955. J. FRIMODT-MØLLER*. *Bull WHO*, No. 1–2, 1960, 22: 61–170. (*Union Mission Tuberc. San., Arogyavaram, South India)

This report deals with the findings of 4 consecutive rounds of mass roentgenographic surveys and tuberculin studies carried out in a relatively small area in South India between 1950 and 1955. The observation period averaged 3½ years. During this period approximately 40,000 were tuberculin tested, and 25,000 were retested at least once. A total number of 63,000 films were taken. Of the persons who had not moved, 93 per cent were examined at least once. In children the number of tuberculin reactors to small doses was low, while the number of reactors to the large doses was remarkably high. Under such conditions the tuberculin test is not of much practical value as a tool for selecting persons for BCG vaccination. On the basis of the repeated tuberculin testing surveys, it is estimated that the infection rate may be 2 to 4 per cent per year. The prevalence of bacillary tuberculosis was between 2.3 and 4.1 per 1,000. Compared with findings in neighboring districts, the prevalence figures in the area under study were definitely low. The annual incidence of new active or probably-active cases of pulmonary tuberculosis following a normal chest roentgenogram was 0.46 per 1,000 in males and 0.41 in females. It is noteworthy that the attack rate was lower in young people than in elderly people. New active or probably active cases developed only in persons showing initial reactions of 10 mm. or more to 5 TU. The tuberculosis mortality rate before the investigation began was probably 200 or more per 100,000. During the 3 intervals between the 4 rounds of mass surveys, when full access to hospital and modern drug treatment was available, the rate was 64, 46, and 21, respectively. This dramatic fall in less than 4 years must be ascribed to the detection of the cases in earlier stages of the disease and the wide use of antimicrobials.
—H. ABELES

Tuberculosis in Rural India. Leading Article. *Tubercle*, June, 1960, 41: 224–227.

In 1950 the World Health Organization Tuberculosis Research Office, in cooperation with the Government of India and the Union Mission Tuberculosis Sanatorium, Arogyavaram, set up a Field Research Center at Madanapalle in South India to investigate the prevalence of tuberculosis in a rural population and the yearly incidence of fresh infections. It was also intended to find out whether it was possible within a few years to reduce the level of infection in such a community by isolation and treatment of infectious patients and BCG vaccination of the uninfected. The most important findings of the investigation may be briefly summarized as follows: a high prevalence of tuberculin sensitization, probably largely by mycobacteria other than *M. tuberculosis;* a re-

markably low prevalence of roentgenographically manifest tuberculosis; a low attack rate with an age pattern similar to that now found in many European and North American countries; and no apparent benefit from BCG vaccination. The greatest difference was seen in those less than 20 years old, in whom the incidence of "active" and "observation" cases was 1.4 in the vaccinated and 0.3 in the unvaccinated, the difference not being statistically significant. This apparent lack of protection from BCG vaccination as carried out in this particular investigation is interesting and disturbing. It is important that it should be realized, from the experience in Madanapalle, that BCG vaccination campaigns, so widely carried out throughout the world, may not have the beneficial effect in preventing tuberculosis that had been hoped for and confidently expected.

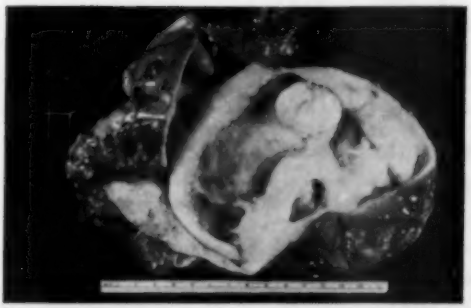
The Madanapalle investigation is of considerable value and it has been reported fully, with proper regard both to the problems of obtaining accurate information on large-scale epidemiologic studies, and the detailed analysis of what has been obtained. It is through investigations such as this, carried out in many other countries, that it will finally become possible to employ properly the technical advances of the past 15 years in order to eliminate tuberculosis.—M. J. Small

Successful Mass Treatment of Tuberculosis at Home. Report by Research Group in India. *Indian Med J*, April, 1960, 4: 91–93.

The results of a carefully organized study in Madras, India, point to the successful treatment of tuberculosis on a mass basis without hospitalization. Two comparable groups of 98 patients treated at home and 97 in a sanatorium were analyzed following 12 months of isoniazid and PAS therapy. None had had previous chemotherapy and 80 in each group had organisms susceptible to both drugs. After 1 year of chemotherapy there was little difference between the 2 groups: only 10 per cent in each group had active disease confirmed by bacteriologic examination. It was most surprising that the obvious dietary disadvantage of those treated at home apparently had no deleterious effect. Cooperation was actually more evident in those treated at home, and an unexpected advantage seen in this group was the much lower incidence of domestic problems.
—E. A. Rouff

The Treatment of Pulmonary Tuberculosis at Work: A Controlled Trial. Report from the Research Committee of the Tuberculosis Society of Scotland*. *Tubercle*, June, 1960, 41: 161–170. (*Edinburgh, Scotland)

One hundred and fifteen patients with pulmonary tuberculosis of mild degree were allocated by random selection to chemotherapy plus bed rest or chemotherapy at work. Any patient with newly diagnosed pulmonary tuberculosis could be admitted to the trial if the clinician thought it would be justifiable to treat him at work—provided that if sputum were present it was negative on microscopy or on culture, and that there was no cavity with a diameter greater than 2 cm. Forty-nine per cent of the bed-rest group had positive cultures from sputum, laryngeal swab, or gastric lavage specimens, compared with 35 per cent in the group at work. Both groups received 5 gm. of sodium PAS plus 100 mg. of isoniazid, usually combined in cachet form, twice daily for 6 months, and thereafter at the discretion of the clinician. Only 2 patients in the trial received no more than 6 months of chemotherapy—both in the working group. After 2 years' observation, those treated at work had done as well as those who had bed rest. Treatment of pulmonary tuberculosis at work should be confined to the highly cooperative patient who is unlikely to be a source of danger to others. The clinician must be on the alert to detect any failure to take with regularity the full dose of the drugs prescribed. If the patient proves unreliable he should be admitted to hospital in order to ensure his compliance with the full chemotherapy regimen on which his future depends.—M. J. Small



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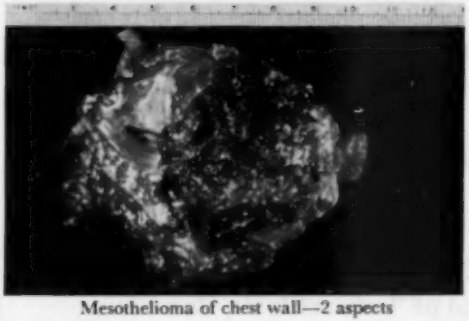

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Starred and unstarred specialists in allergy, bacteriology, gastroenterology, hospital administration, industrial medicine, OALR, OBG, orthopedic surgery, physical medicine—one each	9	2.06
No information	17	3.89
	350	80.19
Institutional subscribers	86	19.72
	436	99.91

MAJOR PROFESSIONAL INTEREST

	No.	%
General private practice	22	5.04
Primary interest in internal medicine and pulmonary diseases	170	38.99
GP with interest in pulmonary diseases	24	5.50
Physician in advanced training	17	3.89
Full-time hospital staff	76	17.43
Full-time medical-school staff	7	1.60
Hospital administration	2	.45
Special research	1	.22
General public-health practice	10	2.29
Retired	3	.68
Not in practice	1	.22
No information	17	3.89
	350	80.19
Institutional subscribers	86	19.72
	436	99.91

AGE OF PHYSICIAN

	No.	%
No information	17	3.89
No age given	1	.22
30–34	10	2.29
35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
50–54	51	11.69
55–59	37	8.48
60–64	35	8.02
65 and over	36	8.25
	350	80.21
Institutional subscribers	86	19.72
	436	99.93

POPULATION

	No.	%
Under 10,000	56	12.84
10–25,000	39	8.94
25–50,000	44	10.10
50–100,000	38	8.71
100–200,000	32	7.33
200–500,000	48	11.00
500–1,000,000	39	8.94
Over 1,000,000	54	12.38
	350	80.24
Institutional subscribers	86	19.72
	436	99.96

BREAKDOWN OF INSTITUTIONAL SUBSCRIPTIONS

Federal Hospital		General Hospital		Special Hospital		Laboratory		Medical Schools		College University		Society Library		Population	
3		2		5		2		0		1		0		Under 10,000	
3		2		1		0		0		1		0		10–25,000	
0		2		4		0		3		2		1		25–50,000	
1		1		1		0		1		2		2		50–100,000	
2		0		1		1		0		0		2		100–200,000	
3		6		0		0		0		1		4		200–500,000	
4		3		2		0		0		1		0		500–1,000,000	
2		4		1		1		2		0		6		Over 1,000,000	
18	4.12%	20	4.58%	15	3.44%	4	.91%	6	1.37%	8	1.82%	15	3.44%	86	19.72%

Percentages figured only to second decimal point

See inside front cover for subscription rate

DIFCO LABORATORY PRODUCTS

BIOLOGICS CULTURE MEDIA REAGENTS

Media for Standard Methods
Culture Media *Dehydrated and Prepared*
Microbiological Assay Media
Tissue Culture and Virus Media
Bacterial Antisera and Antigens
Diagnostic and Serological Reagents
Sensitivity Disks Unidisks
Peptones Hydrolysates Amino Acids
Enzymes Enrichments Dyes Indicators
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over 60 years' experience
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UNIFORMITY STABILITY ECONOMY

Complete Stocks *Fast Service*
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EMERSON
Post-op Suction Pump

1. Very high volume.
   Aspirates 30 liters per minute at a setting of 10 centimeters of water.
2. Low, constant pressure.
   Does not climb appreciably even with tubes wholly obstructed.

Designed for continuous service. May be adapted for 2 patients at once with 2 tubes to each. ✱ ✱ Available in two styles — the "old favorite" or a new, slightly more compact model.

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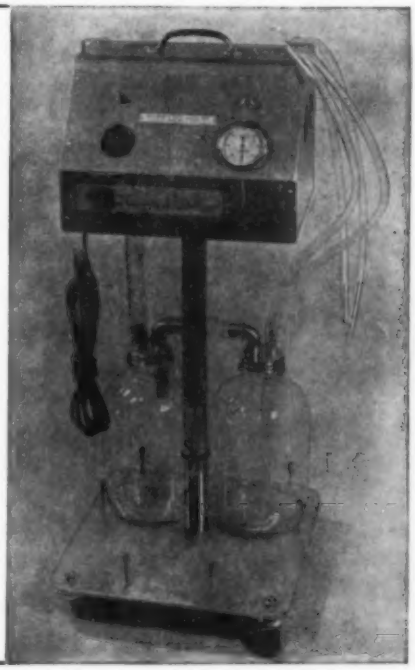


SIMPLIFIES TUBERCULIN TESTING
STERNEEDLE* GUN

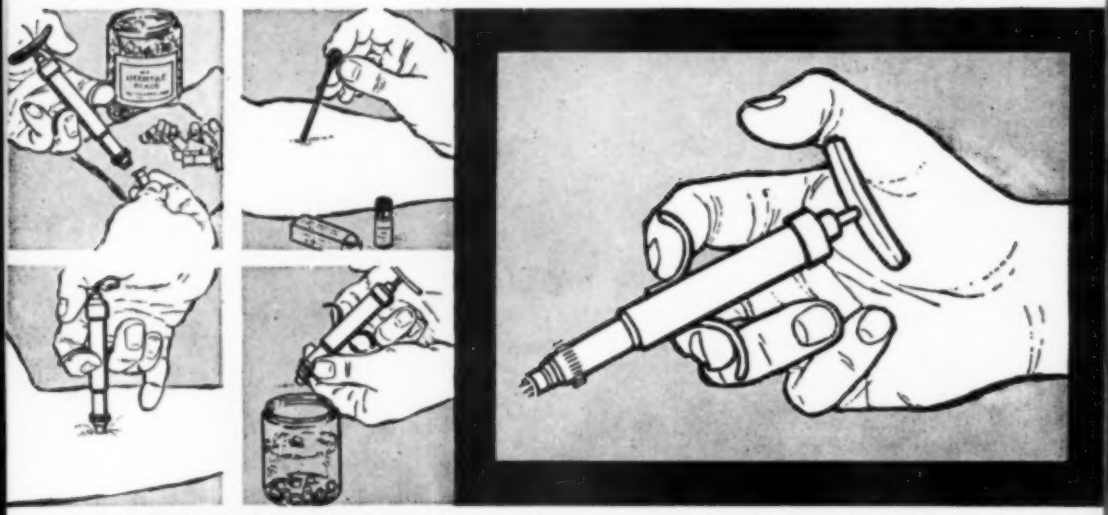
The Automatic TB Case-Finding Device for the Heaf TUBERCULIN TEST

The STERNEEDLE gun is an improved multiple puncture apparatus for rapid intradermal tuberculin testing. Based on the Heaf principle of multiple puncture, six needles contained within the STERNEEDLE cartridge mechanically and painlessly penetrate the skin through a previously applied film of concentrated PPD. Needle puncture and withdrawal is automatic.

ADVANTAGES...

- **Four (4) times faster than Mantoux.**
- **Faster and more reliable than Patch test.**
- **Performs up to 400 tests per hour.**
- **Detects reactors to 5 TU Mantoux and some 100 TU reactors.**
- **No dressing required.**
- **Accurate interpretation, without measurement. Averages 250 readings per hour.**
- **Each test is uniform—puncture of 1 mm. depth and needle withdrawal is automatic.**
- **No special skill required.**
- **Painless. Ideal for testing children.**
- **Eliminates hazardous and uncertain flame sterilization.**
- **Disposable needle cartridges ELIMINATE CROSS-INFECTION.**
- **Low cost STERNEEDLE cartridges may be resterilized.**

Note: CONCENTRATED TUBERCULIN PPD 'CONNAUGHT' is used for the STERNEEDLE test. Available in 1 cc vials (sufficient for as many as 200 tests).



Procedure for STERNEEDLE TUBERCULIN TEST:

Top, left... Slip needle cartridge on gun with plastic applicator. Remove applicator.

Top, right... Place a drop of tuberculin PPD on cleansed forearm.

Bottom, left... Place gun on drop of tuberculin PPD. Pressure and release of handle automatically triggers and retracts the needles.

Bottom, right: Disposable needle cartridges and applicators may be washed and sterilized for reuse, if desired.

Write for literature. Personal demonstration may be arranged.

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THE Panray CORP.

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